Atty. Dkt. No. 045413-0110

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Jonathan A. ELLMAN et al.

Title: PHARMACOPHORE RECOMBINATION FOR THE IDENTIFICATION OF SMALL MOLECULE DRUG LEAD COMPOUNDS

Appl. No.: 10/029,304

Filing Date: 12/28/2001

Examiner: Jon D. Epperson

Art Unit: 1639

DECLARATION UNDER RULE 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Clinton A. Krueger, declare as follows:

1. I am a chemist and have worked in the biopharmaceutical industry since 1999, most recently at Celera Genomics in South San Francisco. I received a B.S. in Chemistry from Bemidji State University in 1992 and a Ph.D in Organic Chemistry from Boston College in 1999.

2. Prior to Celera, I worked at Discovery Partners International (formerly Axys Pharmaceuticals) where I initiated and managed the synthesis of numerous solution-phase as well as solid-phase compound libraries. Illustrative examples of my published work include:

Irving, M.; **Krueger, C. A.**; Wade, J. V.; Hodges, J.; Leopold, K.; Collins, N.; Chan, C.; Shaqair, S.; Shornikov, A.; Yan, B. "High-Throughput Purification of Combinatorial Libraries II: Automated Separation of Single Diastereomers from a 4-Amido-pyrrolidone Library Containing Intentional Diastereomer Pairs" *J. Comb. Chem.* **2004**, *6*, 478-486.

Cournoyer, J. J.; Fang, L.; **Krueger, C. A.**; Wade, J. V.; Yan, B. "The Application of Single Bead FTIR and Color Test for Reaction Monitoring and Building Block

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Validation in Combinatorial Library Synthesis" Chemical Analysis, Vol 163, Wiley Interscience, 2004, Chapter 3.

Yan, B.; Fang, L.; Irving, M.; Zhang, S.; Boldi, A. M.; Woolard, F.; Johnson, C. R.; Kshirsagar, T.; Figliozzi, G. M.; **Krueger, C. A.**; Collins, N. "Quality Control in Combinatorial Chemistry: Determination of the Quantity, Purity, and Quantitative Purity of Compounds in Combinatorial Libraries" *J. Comb. Chem.* 2003, 5, 547-559.

Yan, B.; Fang, L.; Irving, M. M.; Zhao, J.; Liu, D.; Cournoyer, J. J.; Figliozzi, G. M.; Woolard, F. X.; **Krueger, C. A.** "Analytical Methods for Optimization and Quality Control of Combinatorial Synthesis" Combinatorial Chemistry Methods and Protocols – Methods in Molecular Biology, ed. Lisa Bellavance, Humana Press, 2001, Part I, Chapter 11.

3. I have read the above-identified patent application, and believe that the subject matter of the claimed invention is in an area of my expertise.

4. I understand that the captioned application includes claims to a library, wherein each member of the library has the chemical structure CTBF-S-S-R^8 wherein R^8 is a straight chain alkyl of 1 to 10 carbon atoms that is substituted with an amino and CTBF means a candidate target-binding fragment which is a small organic moiety. I find the claimed library sufficiently described in paragraphs 148 (general description of R^8) and 221 (general description of the disulfide containing library) of US 2002/0115107. Because of the presence of a common feature, -S-S- R^8, this library is distinguishable from others such as those described by Roland E. Dolle, Comprehensive Survey of Combinatorial Library Synthesis: 1999, *Journal of Combinatorial Chemistry* 2: 383-433 (2000), a copy of which is attached.

5. I have read and understand U.S. Patent No. 6,552,060. This patent covers compounds of the formula R^1-S-S-R^2 for treating diseases associated with abnormal cellular proliferation or abnormal apoptosis. These compounds are described as asymmetric disulfides because R^1 and R^2 are not identical and have divergent functionalities.

6. The asymmetric disulfide compounds described by the '060 patent are believed to interact with thioredoxin and/or thioredoxin reductase. Thioredoxin is a dual cysteine-containing protein involved in thiol-redox processes in cells. The cysteine pair in thioredoxin is kept in a reduced state by an enzyme, thioredoxin reductase. Figure 5A-C depicts proposed reaction schemes for an asymmetric disulfide R^1-S-S-R^2 with the proteins thioredoxin and thioredoxin reductase. Figure 5A-C does not depict mixtures of compounds having the formula R^1-S-S-R^2.

7. The asymmetric disulfide compounds of the '060 patent were made using parallel combinatorial chemistry (col. 22, lines 49-53). This kind of synthesis makes each compound

in its own reaction vessel and thus relies on automation to achieve increased synthetic productivity.

8. Given the nature of the compounds disclosed by the '060 patent, I do not believe that persons skilled in the art would have been motivated to combine the asymmetric disulfide compounds together. Because of the potential for disulfide exchange, the inventors of the '060 patent likely elected parallel synthesis to minimize the difficulties that would have been encountered using alternative combinatorial methods. Not all classes of compounds are amenable to all combinatorial methods. Having invested the time and effort to make the asymmetric disulfide compounds using parallel synthesis, it is unlikely that a person skilled in the art would take these individual compounds and combine them given that testing pure individual compounds is the most quantitative and robust method of screening that was then known. See generally, Bruce A. Beutel, "Chapter 22: Strategies for Screening Combinatorial Libraries", Combinatorial Chemistry and Molecular Diversity in Drug Discovery, Wiley-Liss, Inc. (1998).

9. I understand one issue in the prosecution of the above captioned application is whether a primary amine is essentially equivalent to a hydroxy or an amine such that a person skilled in the art upon seeing a hydroxy or chloro-containing compound would be motivated to make the amine counterpart with a reasonable expectation of making a compound with similar properties. I understand that pages 19-23 of the book by Richard B. Silverman entitled "The Organic Chemistry of Drug Design and Drug Action" are cited to support the proposition detailed in paragraph 9. I have read and understand the Silverman excerpt and respectfully disagree.

10. The term "isostere" was coined to describe an observation that certain atoms, groups, radicals, and molecules have similar physiochemical properties. Silverman in Table 2.2 (reproduced below) describes the so-called classical isosteres in which he groups together the isosteric definitions proposed by Grimm based on hydride displacement and by Erlenmeyer based on peripheral electrons.

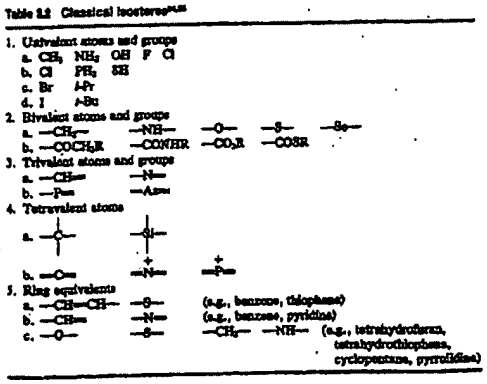


Table 2.2 Classical Isosteres[a,b]

1. Univalent atoms and groups
 a. CH_3 NH_2 OH F Cl
 b. Cl PH_2 SH
 c. Br i-Pr
 d. I t-Bu
2. Bivalent atoms and groups
 a. $-CH_2-$ $-NH-$ $-O-$ $-S-$ $-Se-$
 b. $-COCH_2R$ $-CONHR$ $-CO_2R$ $-COSR$
3. Trivalent atoms and groups
 a. $-CH=$ $-N=$
 b. $-P=$ $-As=$
4. Tetravalent atoms
 a. $-\overset{|}{\underset{|}{C}}-$ $-\overset{|}{\underset{|}{Si}}-$
 b. $-C\equiv$ $-\overset{+}{N}=$ $=\overset{+}{P}=$
5. Ring equivalents
 a. $-CH=CH-$ $-S-$ (e.g., benzene, thiophene)
 b. $-CH=$ $-N=$ (e.g., benzene, pyridine)
 c. $-O-$ $-S-$ $-CH_2-$ $-NH-$ (e.g., tetrahydrofuran, tetrahydrothiophene, cyclopentane, pyrrolidine)

11. Isosteres were first described by Langmuir in 1919. See George A. Patani and Edmond J. LaVoie, Bioisosterism: A Rational Approach in Drug Design, *Chem. Rev.* **96**: 3147-3176 (1996), page 3148, first column. A table showing Langmuir's isosteres is reproduced below. According to Langmuir, the Cl^- can be isosteric with S^{2-}, Ar, K^+ and Ca^{2+}.

Groups of Isosteres as Identified by Langmuir

groups	isosteres
1	H^-, He, Li^+
2	O^{2-}, F^-, Ne, Na^+, Mg^{2+}, Al^{3+}
3	S^{2-}, Cl^-, Ar, K^+, Ca^{2+}
4	Cu^{2-}, Zn^{2+}
↓	↓
8	N_2, CO, CN^-
9	CH_4, NH_4^+
10	CO_2, N_2O, N_3^-, CNO^-
↓	↓
20	MnO_4^-, CrO_4^{2-}
21	SeO_4^{2-}, AsO_4^{3-}

12. In 1925, Grimm extended the concept of isosteres using the Hydride Displacement Law. A table showing Grimm's isosteres is reproduced below (each vertical column represents a group of isosteres). See Patani and LaVoie, page 3148, second column. According to Grimm, hydroxy can be an isostere for amino.

Grimm's Hydride Displacement Law

C	N	O	F	Ne	Na
	CH	NH	OH	FH	–
		CH_2	NH_2	OH_2	FH_2^+
			CH_3	NH_3	OH_3^+
				CH_4	NH_4^+

13. In 1932, Erlenmeyer modified Grimm's definition for isosteres by basing it on whether peripheral layers of electrons can be considered identical. A table showing Erlenmeyer's isosteres is reproduced below. See Patani and LaVoie, page 3148, second column. According to Erlenmeyer, Cl can be an isostere for Br, I, SH, and PH_2.

Isosteres Based on the Number of Peripheral Electrons

no. of peripheral electrons				
4	5	6	7	8
N^+	P	S	Cl	ClH
P^+	As	Se	Br	BrH
S^+	Sb	Te	I	IH
As^+		PH	SH	SH_2
Sb^+			PH_2	PH_3

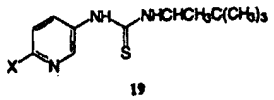
14. The different classifications by Langmuir, Grimm and Erlenmeyer show that which moieties can be considered isosteres depend on which definition is being used. As the

different isostere tables reproduced above show, there is no consensus that an amino is an isostere for either chloro or hydroxy.

15. The term "bioisosterism" takes the isosteric concept one step further by requiring that some biological property be similar. Typically, medicinal chemists desire to keep one or more biological properties constant while modifying others. An iterative approach, this method is used to take lead compounds and turn them into drug candidates. Most medicinal chemist consider the use of bioisosterism an art because the concept is qualitative and intuitive. See Patani and LaVoie, page 3147, second column. The Silverman excerpt supports this view and states that modifying some property of a compound is the intent behind bioisosterism. As stated by Silverman on page 21, "in making a bioisosteric replacement, one or more of the following parameters **will** change: size, shape, electronic distribution, lipid solubility, water solubility, pKa, chemical reactivity and hydrogen bonding (emphasis added)." As a result, even if one were to accept the proposition that an amine is isosteric or even bioisosteric with hydroxyl or chlorine, based on the nature of the functional groups alone, a person skilled in the art would not have any expectation that an amine counterpart of a chloro or hydroxy-containing compound would have similar overall properties.

16. My conclusion in paragraph 15 does not contradict with the English translation of Buchi J. and "The Consitution-Effect Relationships from a New Viewpoint" Deutsche Apotheker-Zeitung 1966, pages 1695-1700 (translation is pages 1-29 as I do not read the Buchi publication to stand for the proposition that making isosteric replacements would result in compounds with similar properties. What Buchi in fact states on page 2 is that "***[i]n physical chemistry***, according to the definition of Erlenmeyer and Leo, atoms, ions and molecues are isosteric if their peripheral electron layers can be seen as identical (emphasis added)." Consequently, Buchi then adds that "it **should be possible** to produce effective analogs, since the electronic charge and physical/chemical properties of such molecule should hardly change (emphasis added)."

17. Being similar in the physical chemical sense is not the same as being similar in all respects including biological activity. Depending on what property is being looked at, examples can be found where an amino counterpart of a chloro or hydroxy-containing compound is similar. An example of this is illustrated by Figure 13 and Table 11 of Patani and LaVoie on page3153 where analogs of N-(substituted-3-pyridyl)-N'-alkylthioureas were evaulated as potassium channel openers. In an *in vitro* assay using rat portal vein, the amino, methyl, and chloro analogs all showed similar activity.



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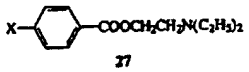
Figure 13.

Table 11. Inhibition of Spontaneous Mechanical Activity in Rat Portal Vein (*in vitro*)

compound	X	maximum fall in SBP[a] (%)	electro-negativity[29]	effective van der Waal's radii[31] (Å)
19a	NH_2	29	2.61	1.79
19b	CH_3	18	2.27	1.80
19c	Cl	27	3.0	1.73

[a] Antihypertensive activity measured as maximum % fall in systolic blood pressure in anesthetized normotensive rat by iv injection.

18. In general, similarity of an *in vitro* property is easier to achieve than similarity of an *in vivo* property. As noted by Patani and LaVoie on page 3151, first column: "[w]hile one may observe retention of activity associated with interaction of a drug with the pharmacophore, bioisosteres may differ dramatically in their *in vivo* efficacy." An example of this is illustrated by Figure 18 and Table 14 of Patani and LaVoie on page 3154, second column where the biological half-lives of para-substituted 2-(diethylamino)ethyl benzoates were evaluated. As it can be seen, the half-life of the amino compound is almost 20 fold longer than the chloro counterpart. This difference is attributed to the electron-withdrawing characteristic of the chloro group likely making the compound more susceptible to hydrolysis.



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Figure 18.

Table 14. Biological Half-Lives of *Para*-Substituted 2-(Diethylamino)ethyl Benzoates

compound	X	σ_p	$t_{1/2}$
27a	NH_2	−0.66	96.29
27b	OCH_3	−0.26	26.75
27c	Cl	0.23	4.94
28d	CN	0.63	1.28

19. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

3/30/2006
Date

Clinton A. Kruger

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Reviews

Comprehensive Survey of Combinatorial Library Synthesis: 1999

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Received June 30, 2000

Continuing with this annual series of comprehensive surveys of combinatorial libraries,[1] the present review captures small molecule libraries for pharmaceutical applications reported in the literature during the year 1999. The total number of libraries published in 1999 was 292. There were 85 citations for libraries describing biologically active agents and 207 citations for library constructs without disclosed biological activity.[2-252] Overall, these numbers are quite similar to those reported in last year's review.[1a] Last year, the first example of an efficacious and orally active compound obtained directly from an optimization library was reported.[253] In addition to new examples of orally bioavailable agents coming from chemical libraries,[8,229,252,101] this year marks another milestone: a 500-member optimization library played a defining role in the identification of a clinical candidate.[66,153] The effort was reported by Agouron Pharmaceuticals in their structure-based rhinoviral 3C-protease inhibitor program. Achievements such as these are worth noting as large capital investments have been made in combinatorial chemical technologies.[254] Today combinatorial synthesis pervades many aspects of drug discovery from lead finding and target validation, lead optimization, to enhancing corporate compound collections.

Including the libraries compiled herein, a total of 975 libraries have been abstracted along with their generic structures in this comprehensive review series,[1] beginning in 1992 when the first publications of libraries began to appear in the literature. An analysis of the data collected in the reviews reveals some interesting statistics and trends in combinatorial chemical research (Figures 1-5). Figure 1A graphically illustrates the number of libraries published during the years 1992-1999 as divided into two broad classifications: (1) chemical libraries for which their synthesis and biological assay data is reported (disclosed biological activity), and (2) chemical libraries for which only their synthesis was reported and no disclosure of biological activity (undisclosed biological activity). The number of reports of biologically active libraries grew at a fairly steady pace. The largest single jump (10-fold) occurred in 1995, with a steep rise occurring in 1998-1999. The 1998 library number of 74 is nearly equivalent to the combined total of the preceding 6 years. The number of biologically active libraries for 1992 through 1999 was 240. In contrast, the number of reports of library synthesis without disclosed biological activity rose at a much more dramatic pace as the nascent field began to take root. In 1992-1994, only 15 libraries of this type in total had been reported, comparable in number to the 12 biologically active libraries reported for the same period. Library citations (without biological data) increased by a factor of 3× in 1995 to 43 libraries. In 1996 library publications of this genre more than doubled (2.5×), held steady for 1997, and then doubled again in 1998 to 247 libraries. Libraries with undisclosed biological activity fell back slightly to 207 libraries in 1999. The total number of libraries in this classification is 735, some 70% more than reports of biologically active libraries. This gap is not too surprising since researchers are anxious to demonstrate new chemical methodologies, while safeguarding the structures of active library members. Figure 1B shows the cumulative

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10.1021/cc000055x CCC: $19.00 © 2000 American Chemical Society
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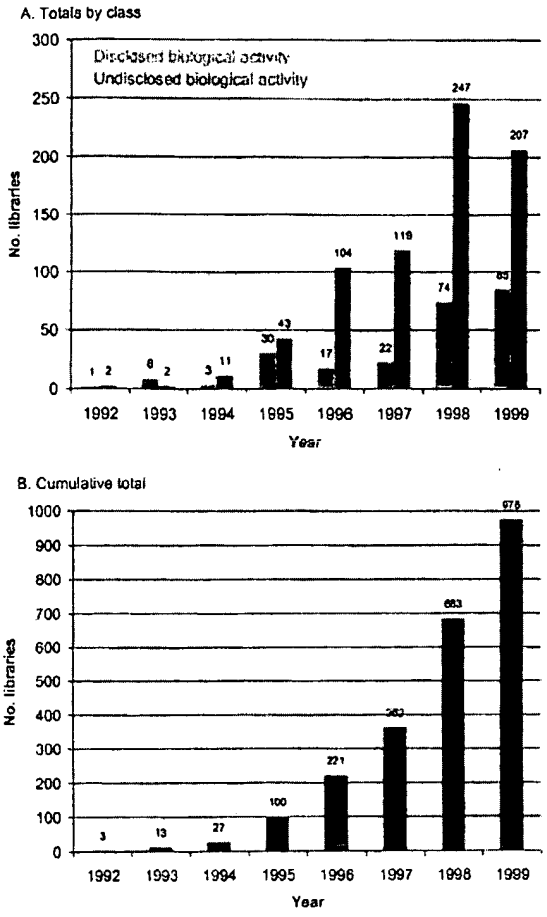


Figure 1. Libraries by major class.

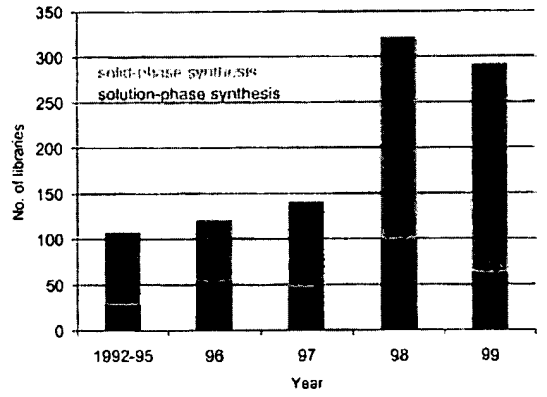


Figure 2. Solid- versus solution-phase for all library constructs (1992–1999).

total of both library classifications, which on balance has increased approximately 1.5× each year.

The early appeal of combinatorial chemistry was creating large discovery-type libraries through synthesis on solid support. In addition to its perceived synthetic advantages, solid-phase synthesis was the overwhelming choice for library construction in 1992–1995 (Figure 2). Some 80% of the libraries produced in this time period were generated on solid support. Solution-phase synthesis surged to 50% of

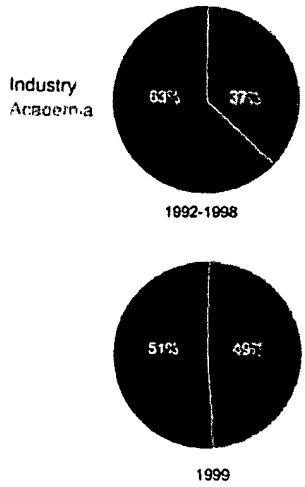


Figure 3. Library contributions by affiliation (1992–1999).

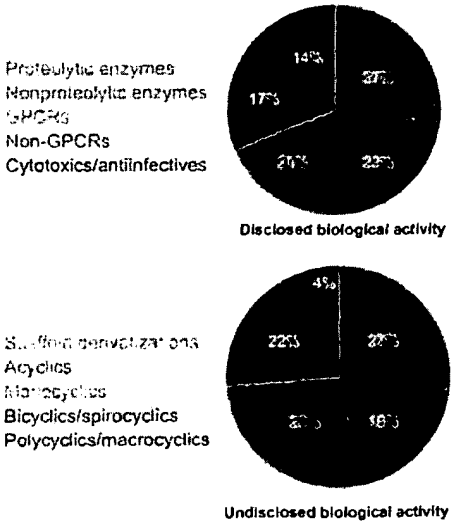


Figure 4. Libraries by subclass (1992–1999).

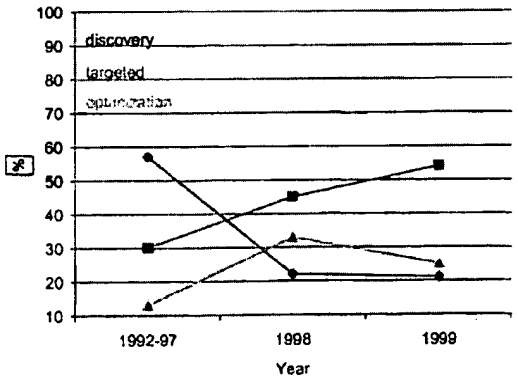


Figure 5. Discovery, targeted, and optimization libraries (biologically active libraries only).

the total reports in 1996. This was led by advances in the development of new solid-phase reagents, scavenger resins, novel fluorous-based separations, and automated liquid–liquid extractions. Publications of solution-phase library

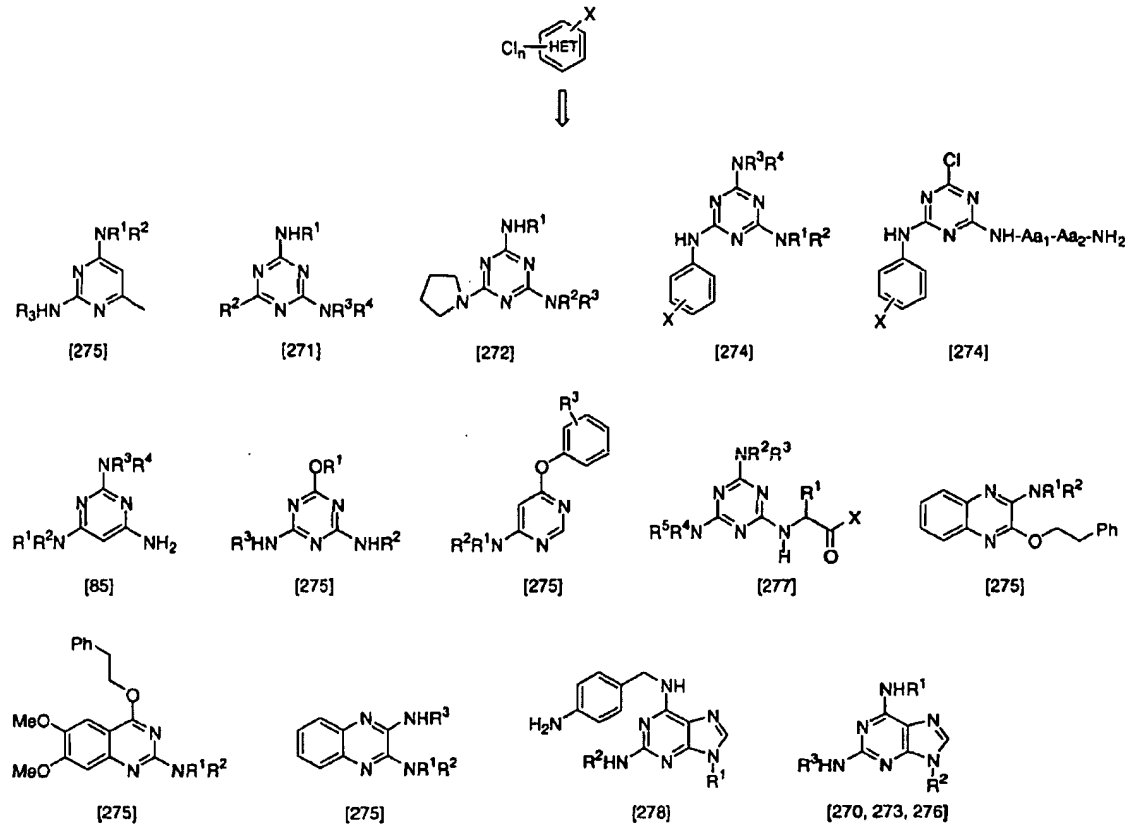


Figure 6. Libraries from polyhalogenated heteroaromatic scaffolds.

synthesis remained steady at ca. 33% in 1997 and 1998, but receded to its 1992−1995 levels (20%) this immediate past year. The data suggest that solid-phase synthesis continues to hold a dominant position in combinatorial synthesis as more and more chemistries are redeveloped on this medium.

Figure 3 indicates the origin of library contributions over the last 8 years, i.e., from the laboratories of academia or industry. In the combined years 1992−1997, two-thirds of the contributions were from industrial laboratories, with this number remaining relatively constant in 1998. This past year library affiliations moved to an industry:academia ratio of 1:1. Overall, pharmaceutical and biotechnology industries appear to be the prevailing players in the game of small molecule combinatorics, motivated by the goal of increasing drug discovery speed and reducing costs. The majority of academic publications showcased new synthetic methodologies.

Figure 4 reveals the breakdown of libraries by subclass. Biologically active libraries are designated into one of five subclasses. These include proteolytic enzymes (27%), non-proteolytic enzymes (22%), GPCRs (20%), non-GPCRs (17%), and cytotoxic and antiinfective agents (14%). Within the proteolytic enzyme subclass, serine proteases, namely the trypsin superfamily, were the most screened molecular targets. For GPCRs, opioid receptors appear to be the perennial favorite, not so much as a serious molecular target, but a convenient demonstration of library utility. Libraries without reported screening data also fall into one of five categories: scaffold derivatizations (27%), acyclic synthesis (19%), monocyclic synthesis (28%), bicyclic and spirocyclic synthesis (22%), and polycyclic and macrocyclic synthesis (4%). A widely used scaffold or template for derivatization is the polyhalogenated heterocycles, e.g., cyanuric chloride and trichoropyrimidine (Figure 6).[270−278] Substituted fluoronitroaromatics have been especially versatile reagents for the construction of mono-, bi-, and macrocycles (Figure 7).[255−269] Many of the classical routes to heterocycles have been reported on solid phase.[290]

Focusing on the 240 biologically active libraries published in 1992−1999, one can readily distinguish between discovery, targeted, and optimization libraries (Figure 5). For the purpose of this discussion, discovery libraries are defined as typically large in size (>5000 members) having no preconceived notions about which molecular target(s) it may be active against. Targeted libraries are biased in their design, defined as those libraries which contain a pharmacophore known to interact with a specific (or family of) molecular target. Optimization libraries are defined as those libraries in which a lead exists and an attempt is being made to improve its potency, selectivity, pharmacokinetic profile, etc. Accordingly, each of the 240 libraries have been examined and binned into one of these three categories. Between the early years 1992−1997, discovery libraries garnered the highest percentage of citations at 57%. This was twice the percentage of targeted libraries and 4 times the reported number of optimization libraries. The number of discovery

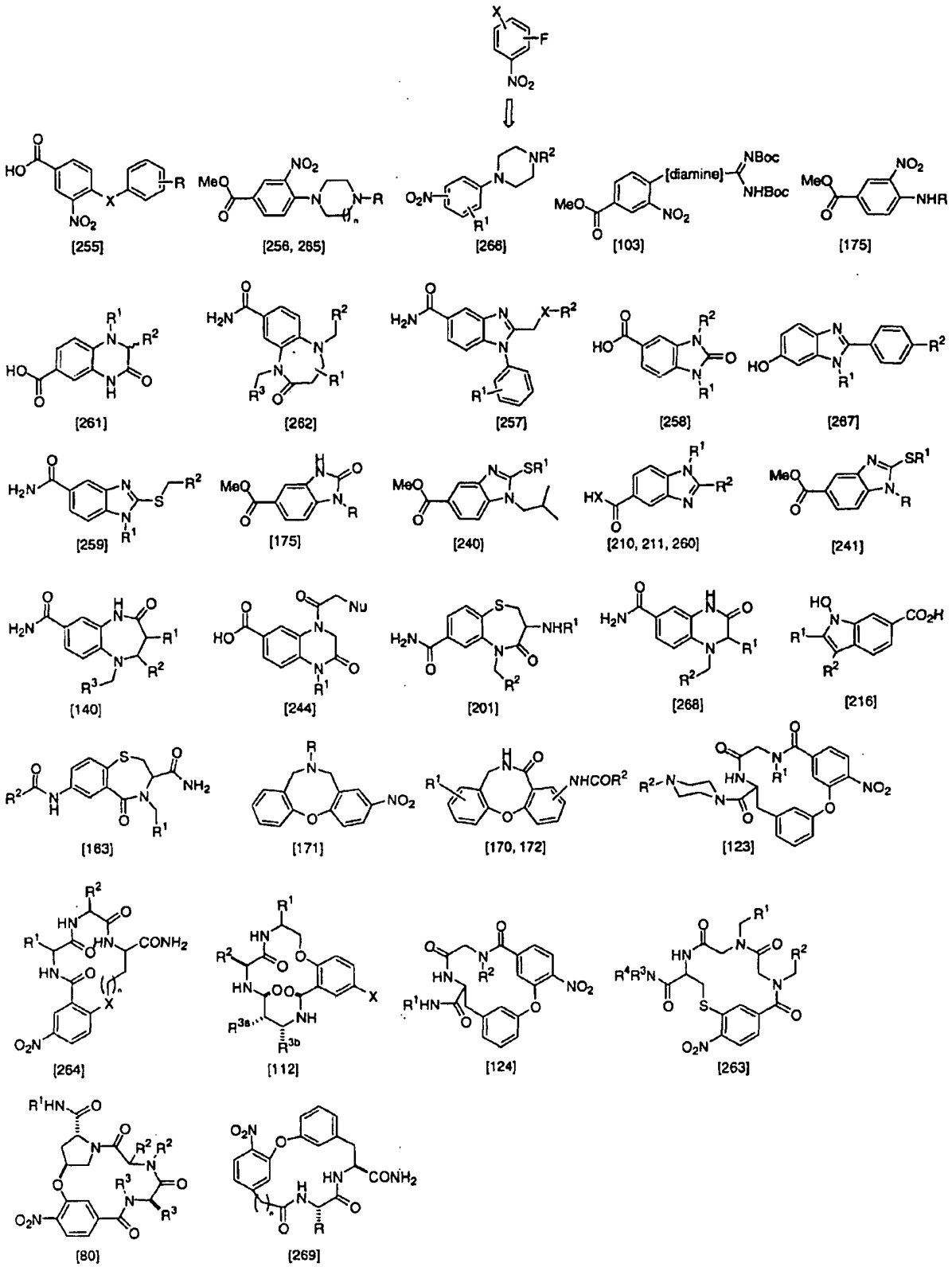


Figure 7. Libraries from fluoronitroaromatic scaffolds.

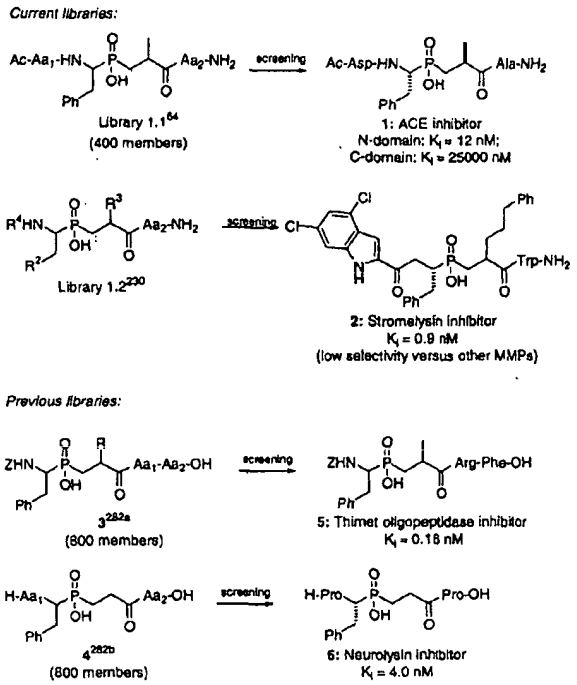


Figure 8. Dive's libraries of phosphinic acids.

libraries has fallen rather significantly in the past two years from its 57% high to now the lowest in the group at 21%. In the same 2 year period, targeted libraries now top the charts, rising from 30% → 45% → 54%. Optimization libraries rose from 7% (1992–1997) to ca. 20% (1999), equal to the number of discovery library disclosures. It is tempting to speculate whether this represents a *true* shift in the way the combinatorial chemistry is being valued and applied in drug discovery, or an artifact of industrial research released for external consumption.[2] Anecdotal evidence from discussions at recent conferences and literature commentaries suggest targeted library collections biased toward a specific class or family of molecular targets and "lead explosion" libraries may be preferred over large discovery-type libraries.[254,279,280] Certainly large libraries offer unique advantages over smaller focused collections providing they can be designed with drug-like properties and screened efficiently and the actives can be readily identified.[281]

One of the criticisms leveled against combinatorial chemistry and which may still slow the acceptance of the technology is that the chemistries generally yield structures that are too peptide-like and contain multiple amide bonds. This is a valid concern due to the known pharmacokinetic liabilities, poor drug-like characteristics, and difficulty in optimizing these types of compounds. Data derived from the biologically active libraries show that, of the libraries reported during 1992–1997, ca. 50% were in fact peptide-based (more than three contiguous amino acid residues). Approximately 70% of the libraries incorporated one or more α-amino acids, and ca. 85% of the libraries contained one or more amide bonds (data not shown). In the combined years 1998–1999, the number of reported peptide libraries fell by more than half to ca. 20%, most likely reflecting a bona fide loss in interest in these types of libraries. The use of α-amino

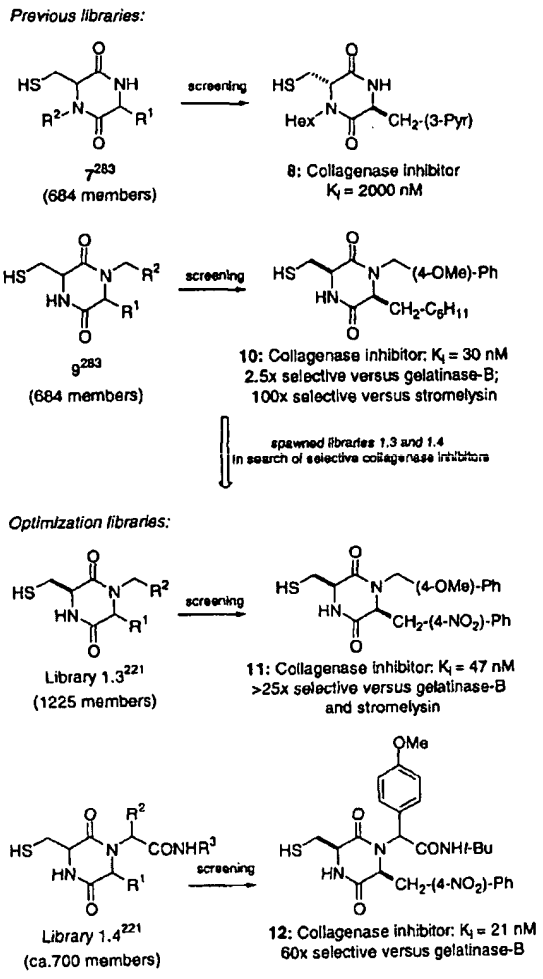


Figure 9. Affymax's thiomethyldiketopiperazine libraries.

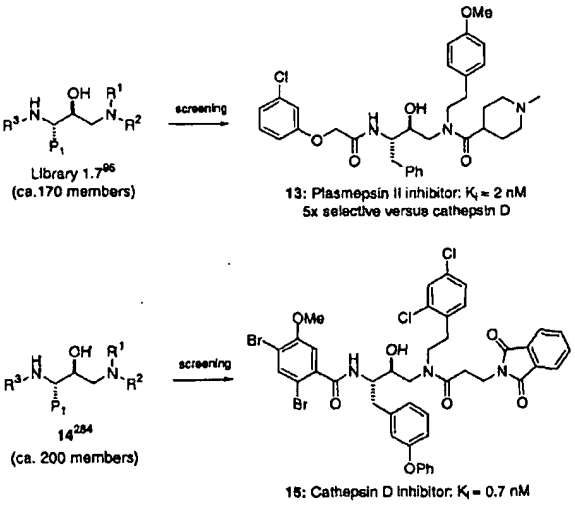


Figure 10. Hydroxyethylamine libraries for cathepsin D and plasmepsin II inhibition.

acids in library construction remains high at ca. 50%, as these synthons represent an excellent source of chiral, low molecular weight diversity elements.

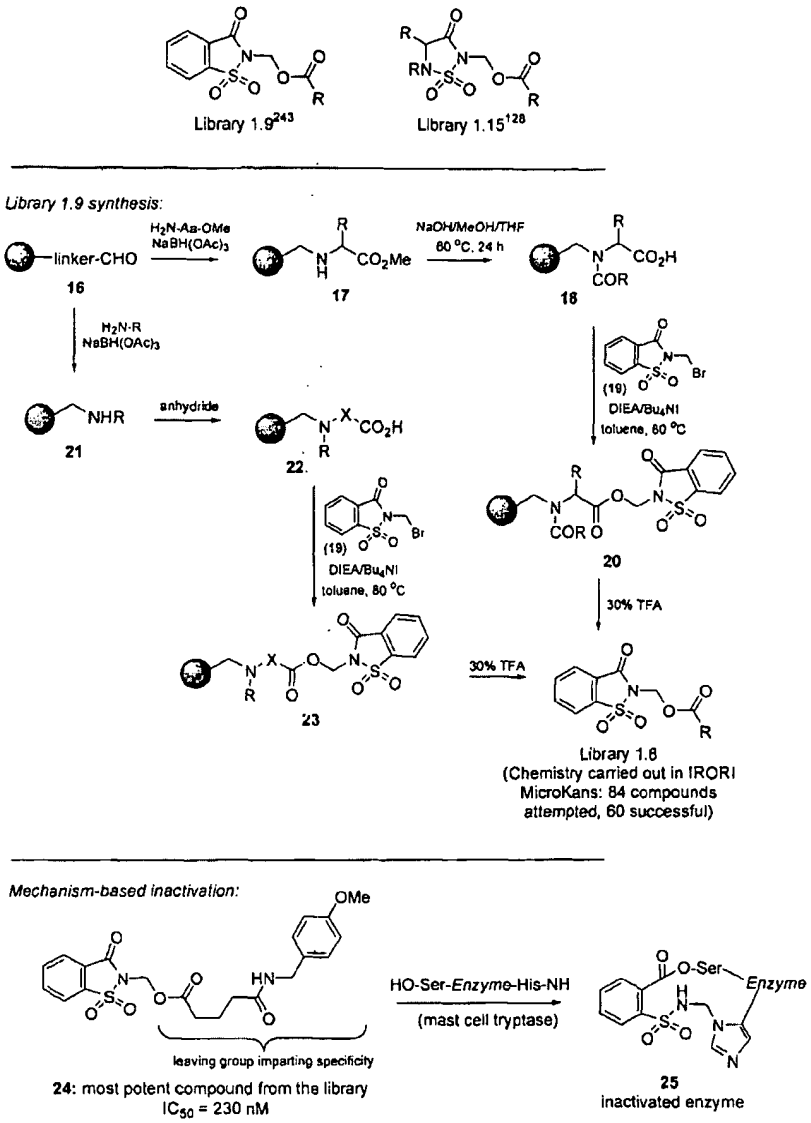


Figure 11. Mechanism-based libraries targeted for (chymo)trypsin serine proteases.

	1992−1997	1998	1999
peptide-based libraries	50%	20%	21%
libraries using ≥ 1 amino acid	70%	55%	53%
libraries containing ≥ 1 amide bond	85%	65%	75%

Finally, the notion that combinatorial synthesis acting *alone* will accelerate drug discovery research has not been borne out by experience over this first decade. Ideology of a single universal library as a source of leads against a plethora of molecular targets, purported by some, is not credible. What is evident is that combinatorial synthesis is an important technology among a suite of technologies that can be brought to bear on solving drug discovery problems.

Library Descriptions

Consistent with the format of previous annual reviews,[1] the abstracted 1999 libraries are sorted into two major categories, libraries with and without associated biological activity. Biologically active libraries are further sorted into five subclasses: proteolytic enzymes (Table 1), nonproteolytic enzymes (Table 2), GPCRs (Table 3), non-GPCRs (Table 4), and cytotoxic and antiinfective agents (Table 5). The name of each library is given, along with its size and affiliation (company name for libraries produced from industry, senior author for libraries reported from academia), as well as the structure of the most active compound from the library. Each library listed in Tables 1−5 is given a library number, e.g., library 2.10 refers to library entry 10 in Table 2. Libraries without accompanying biological data are also segregated into five subclasses. Here each entry is further subdivided as per the mode of synthesis, solid- versus solution-phase synthesis: scaffold derivatization (Table 6a,b), acyclic synthesis (Table 7a,b), monocyclic synthesis (Table 8a,b), bicyclic and spirocyclic synthesis (Table 9a,b), and polycyclic and macrocyclic synthesis (Table 10a,b). The affiliation of each library is provided, along with the number of synthetic examples, range of reported reaction yields, and

a brief description of its synthesis. As indicated previously,[10] the size of the reported library does not necessarily reflect confirmed library size. Single synthetic transformations, phage display, polysaccharide, and polynucleotide libraries, and libraries for applications in material science or other nonpharmaceutical research areas, are not included in the tables.

Libraries Yielding Proteolytic Enzyme Inhibitors

Each of the four broad classes of proteases—metallo- (libraries 1.1−1.5), aspartyl- (libraries 1.6−1.8), serine- (libraries 1.9−1.18), and cysteine proteases (libraries 1.19−1.20)—were targeted for library synthesis (Table 1). As in previous years, mechanism-based design strategies were generally employed to create protease inhibitor libraries. This approach relies on selecting a functional group or pharmacophore known to engage an enzyme's active site residues and building a library around the scaffold in an effort to obtain potent and selective inhibitors.

In a continuation of their research on the preparation of libraries containing the phosphinic acids, a transition-state isostere for metallo-proteinases,[282] Dive and co-workers described the preparation of two new peptide phosphinic acid libraries, 1.1 and 1.2 (Figure 8). Library 1.1 yielded a selective N-domain inhibitor **1** of angiotensin I converting enzyme (ACE).[64] A selective inhibitor **2** of stromelysin (matrix metallo-proteinase-9) was obtained from library 1.2.[230] The structurally related tripeptide phosphinic acid libraries **3** and **4** were first described by Dive in the identification of potent and selective inhibitors **5** and **6** of the metallo-proteases thimet oligopeptidase and neurolysin I.[282] The ACE inhibitor **1** is structurally distinct from inhibitors **5** and **6**, and its discovery is significant in that it is the first agent to discriminate between the catalytic N- and C-domains in this enzyme. The C-domain of ACE catalyzes the hydrolysis of angiotensin I and angiotensin II regulating blood pressure, while the N-domain of ACE is thought to be responsible for the specific hydrolysis of other physiologically important substrates, e.g., Ac-Ser-Asp-Lys-Pro, a negative regulator of hematopoietic stem cell differentiation and proliferation. Currently, marketed ACE inhibitors do not discriminate between the enzyme's two catalytic domains, and thus the selective N-domain inhibitor **1** may prove to be a useful pharmacological tool in understanding the role of the N-domain in vivo.

Affymax reported the synthesis of two thiol-containing diketopiperazine libraries (libraries 1.3 and 1.4; Figure 9).[221] The research group previously disclosed these thiol-containing heterocycles as possessing inhibitory action against the matrix metalloproteinases (MMPs).[283] In the earlier work, potent collagenase inhibitors were discovered, but these lacked selectivity (**7** → **8**; **9** → **10**). It was the goal of the new libraries to enhance this aspect of the series. Selectivity was imparted to the class by incorporating nitrophenylalanine as one of the amino acid monomers, furnishing inhibitors **11**, K_i = 47 nM (>25-fold versus gelatinase B and stromelysin), and **12**, K_i = 21 nM (ca. 60-fold selective versus gelatinase-B).

In a full paper, Ellman described further utility of hydroxyethylamine libraries as inhibitors of aspartyl proteases.[95] A series of optimization libraries (library 1.7, Table 1) were systematically prepared, ultimately furnishing potent inhibitors of plasmepsin, e.g., **13**, although in general, these agents demonstrated weak selectivity against cathepsin D (Figure 10). Throughout the work, particular attention was paid to the physicochemical properties of the libraries and resynthesized compounds as measured against the Lipinski parameters. Earlier work with libraries of this class furnished potent cathepsin D inhibitors (**14** → **15**).[284]

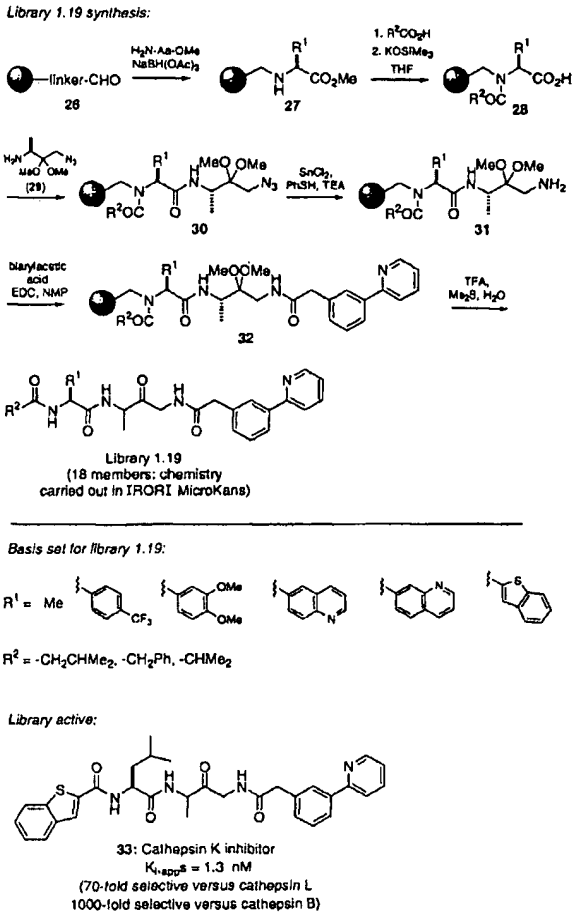


Figure 12. 1,3-Bis(acylamino)-2-butanones library as cysteine protease inhibitors.[239]

Libraries possessing inhibitors of trypsin-like enzymes were reported from several groups. These included δ-ketothiazoles (library 1.10),[3] arylamidines (libraries 1.11 and 1.12),[22,184] benzothiophenes (library 1.13),[114] and aminocyclohexanones (library 1.14).[2] A novel series of amino acid sulfonamides were optimized to yield an orally bioavailable thrombin inhibitor (library 1.18).[252] Two examples of mechanism-based inhibitor libraries of serine proteases leading to covalent adduct formation were reported. These include the benzisothiazolones (library 1.9),[243] yielding inhibitors of tryptase, and thiadiazolidin-3-ones (library 1.15),[128] showing a broad spectrum of affinity for serine proteases with a (chymo)trypsin-like fold (Figure 11).

SmithKline Beecham published on the design and enzymology of a novel class of 1,3-bis(acylamino)-2-butanones

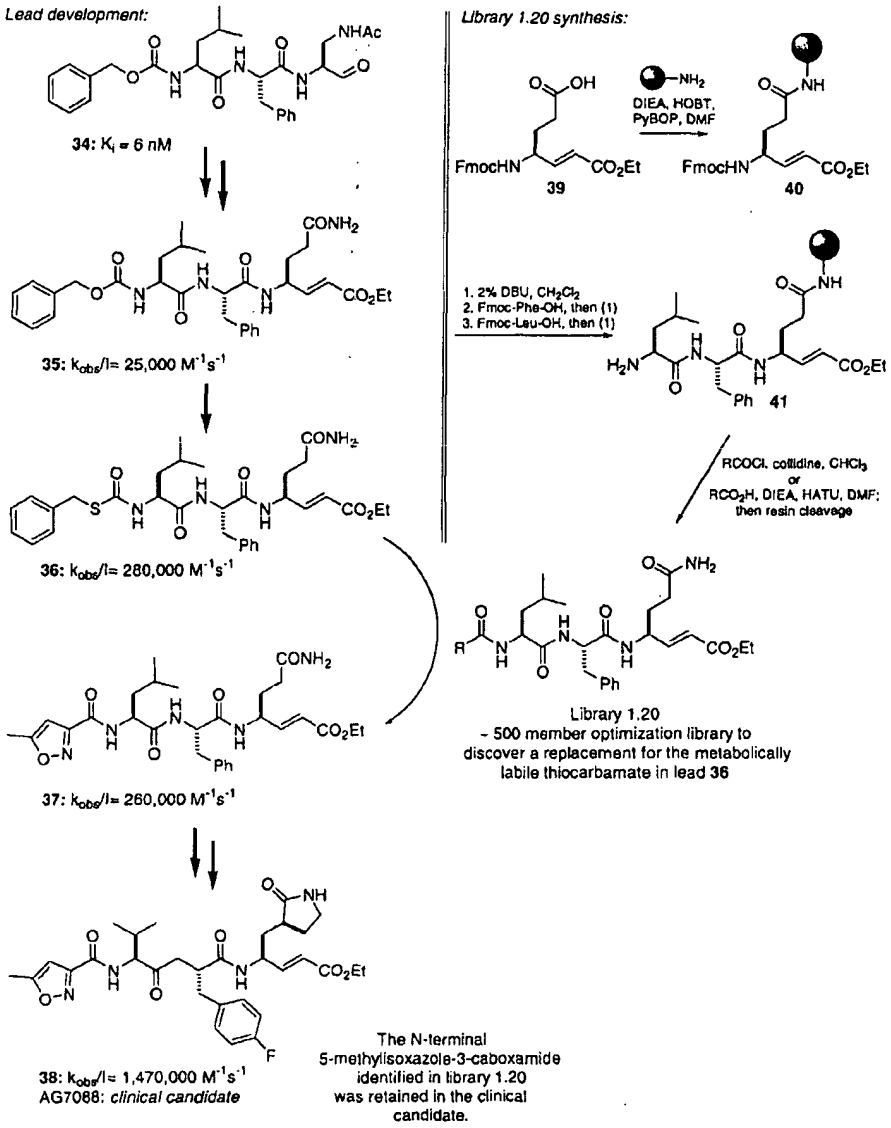


Figure 13. Optimization library 1.20 and the identification of a clinical candidate for human rhinovirus 3C protease.[66,153]

as cysteine protease inhibitors.[239] Their interest in this area is a result of a discovery program aimed at identifying inhibitors of cathepsin K.[285] Cathepsin K is a cysteine protease that degrades collagen at sites of bone remodeling, and inhibitors thereof may represent potential antiosteoporetic agents. In an effort to facilitate the rapid optimization of the 1,3-bis(acylamino)-2-butanone inhibitors, a solid-phase synthesis for this class of compounds was developed (Figure 12). Using the acid labile BAL aldehyde linker on polystyrene resin, the synthesis was initiated via the reductive amidation of amino acid esters onto linker **26**. Acylation of the resulting secondary amine and hydrolysis furnished acid **28**. Coupling **28** to the orthogonal protected azide amine **29** gave resin-bound intermediate **30**. Azide **29** was prepared in solution via a four-step sequence from Boc-alanine methyl ester. Reduction of the azido group in **30**, then acylation with 3-(2-pyridinyl)phenylacetic acid, and acid-mediated cleavage furnished the library compounds. Although optically active synthons were used in the library construction, inspection of the final products showed that epimerization had occurred to some extent. This was thought to take place during the coupling of azide **29** with resin-bound acid and upon the hydrolysis of the dimethyl ketal protecting group. Library synthesis was carried out using the IRORI R_f tags. Evaluation of the library against cathepsins K, L, and B revealed interesting SAR. The library was essentially devoid of cathepsin B activity. This was believed to be due to an unfavorable interaction of the heterobiaryl with an insertion loop present on the S′ side of the enzyme. Cathepsin K had a strong preference for leucine versus phenylalanine at the P_2 position, while cathepsin L showed a slight preference for phenylalanine. The most potent cathepsin K inhibitor was compound **33**: K_i = 1.3 nM, ca. 70-fold selective versus cathepsin L.

A beautiful example of the application of solid-phase chemistry in drug discovery is found in the optimization of

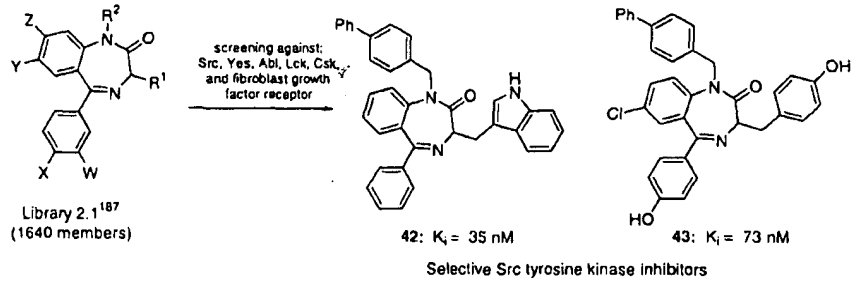


Figure 14. 1,4-Benzodiazepines as inhibitors of Src protein tyrosine kinase.[187]

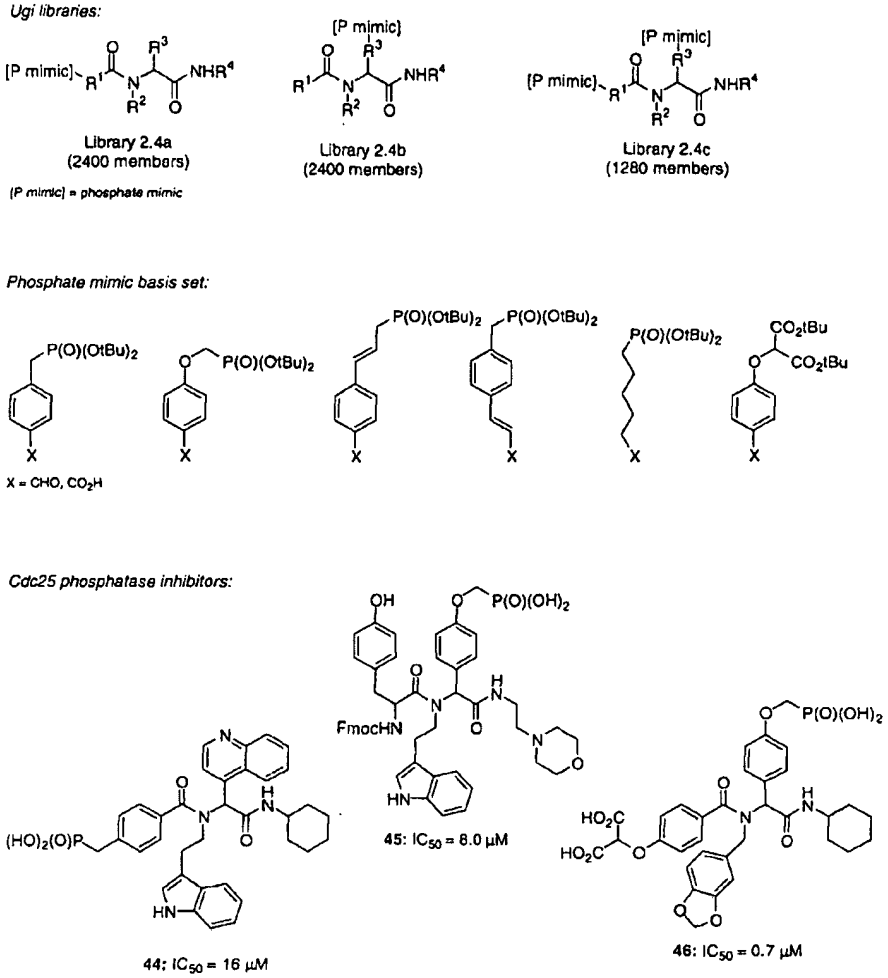


Figure 15. Ugi libraries of Cdc25 phosphatase inhibitors.[14]

irreversible human rhinovirus 3C protease inhibitors leading to a clinical candidate.[66,153] Researchers at Agouron Pharmaceuticals had shown that substrate-based peptide aldehydes, represented by **34**, were potent, reversible inhibitors of 3C protease. Due to the well-known pharmacological limitations of peptide aldehydes as viable drug candidates, the group turned to peptide Michael acceptors as covalent, irreversible inhibitors of the cysteine protease with the belief that the electrophilicity of these agents could be sufficiently modulated through high enzyme specificity. The lead compound **35**, based on the enzyme's P_1 and P_2 specificity preferences, possessed a second-order rate constant k_{obs}/I = 25 000 M^{-1} s^{-1}. Exchange of the N-terminal benzyloxycarbamoyl group in **35** for the benzylthiocarbamoyl group led to a *10-fold increase* in the second-order rate constant (**36**: k_{obs}/I = 280 000 M^{-1} s^{-1}). The rationale for the boost in affinity was provided through analysis of the X-ray crystal structure of inhibitor **36** bound to serotype-2 3C protease. The crystal structure revealed that the thiocarbamate sulfur atom lies deep in the enzyme's S_4 pocket and is in van der Waals contact with the S_4 subsite's Phe residue. This is in contrast to the oxygen analogue **35**. However, there was

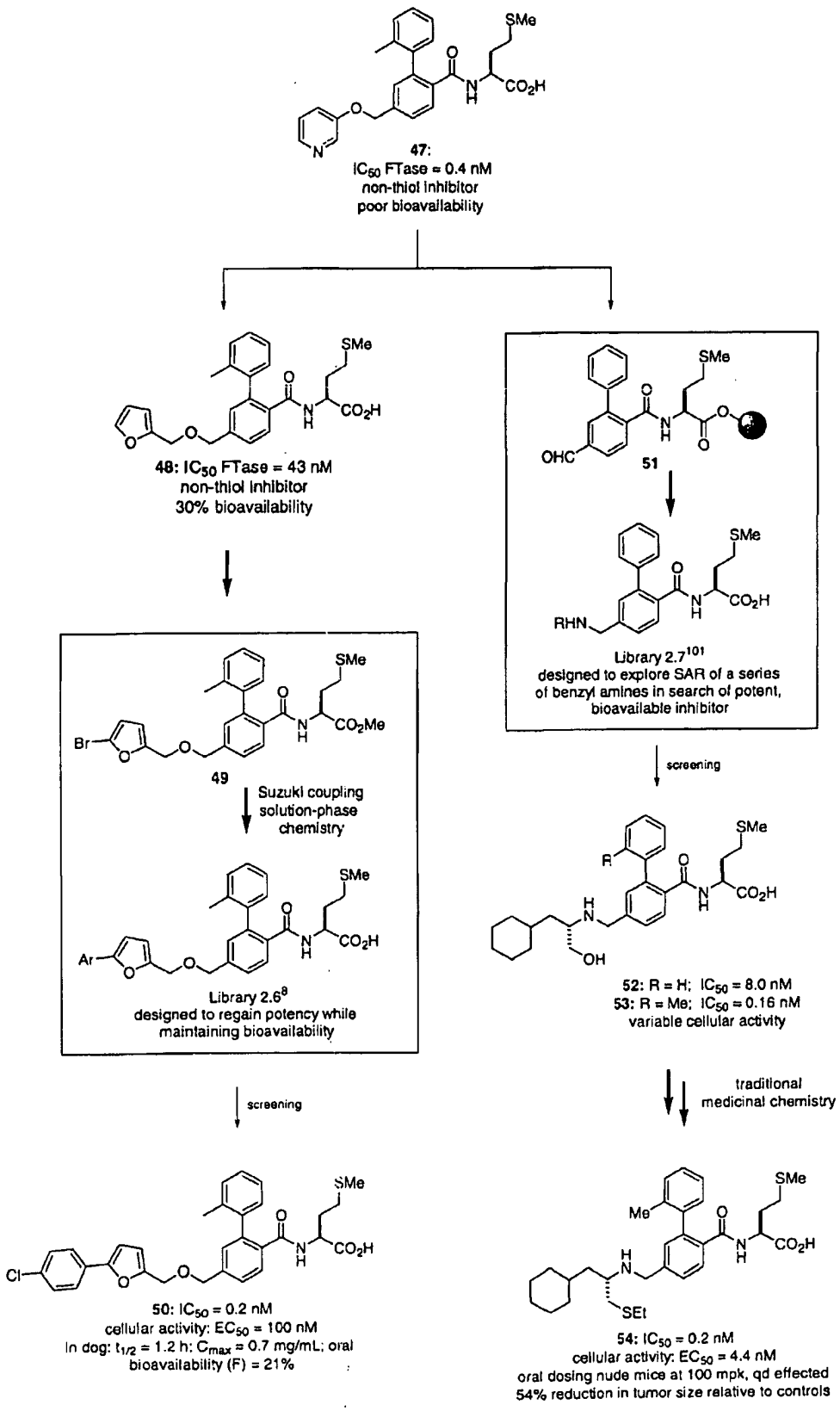


Figure 16. Abbott's FTase libraries.[8,101]

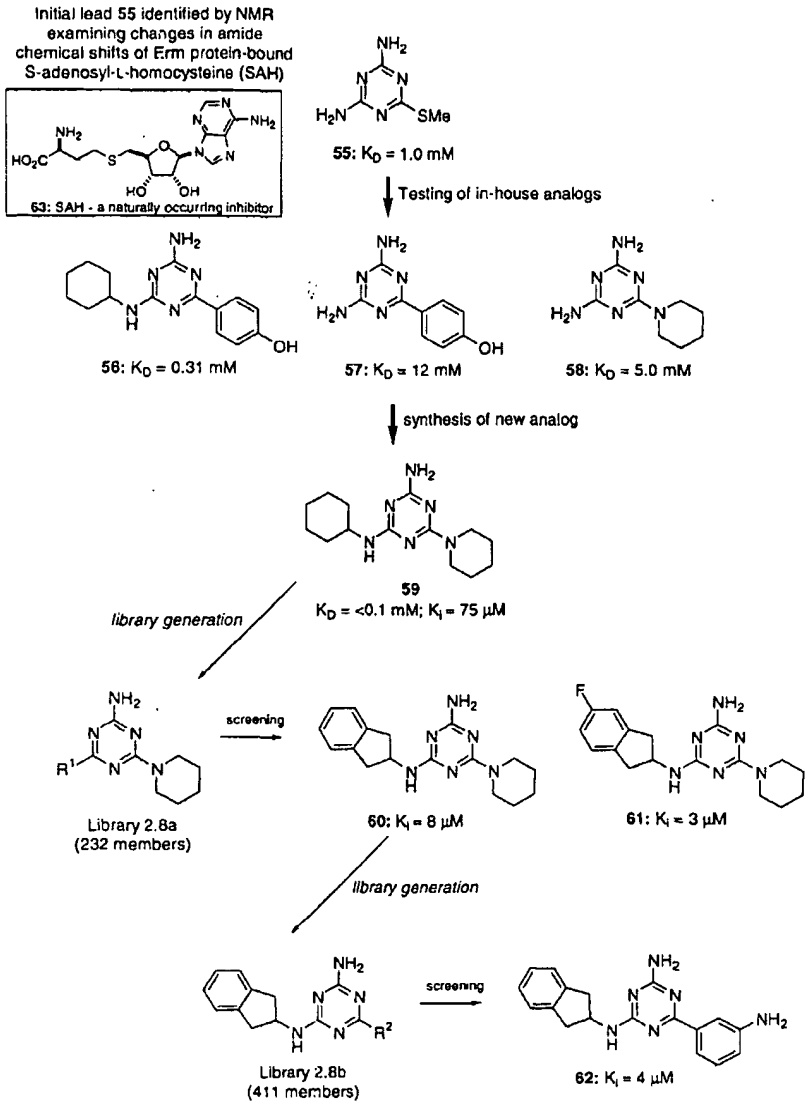


Figure 17. Inhibitors of Erm methyltransferase by NMR and parallel synthesis.[14]

concern that the thiocarbamate moiety would prove to be a metabolic and/or toxicologic liability, and thus an N-terminal surrogate was sought. This was carried out through solid-phase chemical optimization. Library 1.20 was created by attaching glutamic acid analogue **39** to Rink resin (Figure 13). Amide **40** so obtained was deprotected and subjected to amino acid couplings to furnish **41** after Fmoc-deprotection. Amine **41** was a key intermediate derivatized with some 500 acylating reagents to generate the optimization library. Evaluation of library 1.20 using a high throughput assay identified the 5-methylisoxzole-3-carboxyl group as the preferred N-terminal surrogate. This heterocycle was incorporated into the main series (**36** → **37**). Compound **37** (k_{obs}/I = 260 000 M^{-1} s^{-1}) was essentially equipotent with thiocarbamate **36** (k_{obs}/I = 280 000 M^{-1} s^{-1}). Further analogues produced inhibitor **38** (AG7088; k_{obs}/I = 1 470 000 M^{-1} s^{-1}) with reduced peptide character. AG7088 is currently undergoing clinical evaluation for the treatment of rhinoviral-mediated infections, e.g., the common cold. This research effort represents a prime example of the value of combinatorial chemistry (solid-phase synthesis) in lead optimization. In this instance, the N-terminal capping element 5-methylisoxazole-3-carboxamide identified in library 1.20 was retained in the clinical candidate.

Libraries Yielding Nonproteolytic Enzyme Inhibitors

Table 2 lists 19 libraries displaying activity against nonproteolytic-type enzymes. The table is subdivided into kinases and phosphatases (entries 2.1−2.4), transferases (entries 2.5−2.8), reductases and dehydratases (entries 2.9−2.12), and miscellaneous mammalian and nonmammalian enzymes (entries 2.13−2.19).

Benzodiazepine library 2.1, composed of 1640 members and prepared in the Ellman group, was screened against a wide variety of protein tyrosine kinases including Src, Yes, Abl, Lck, Csk, and fibroblast growth factor receptor (Figure 14).[187] Binding was observed only against the Src family (mixed against the peptidic substrate, K_i = 35 μM; noncom-

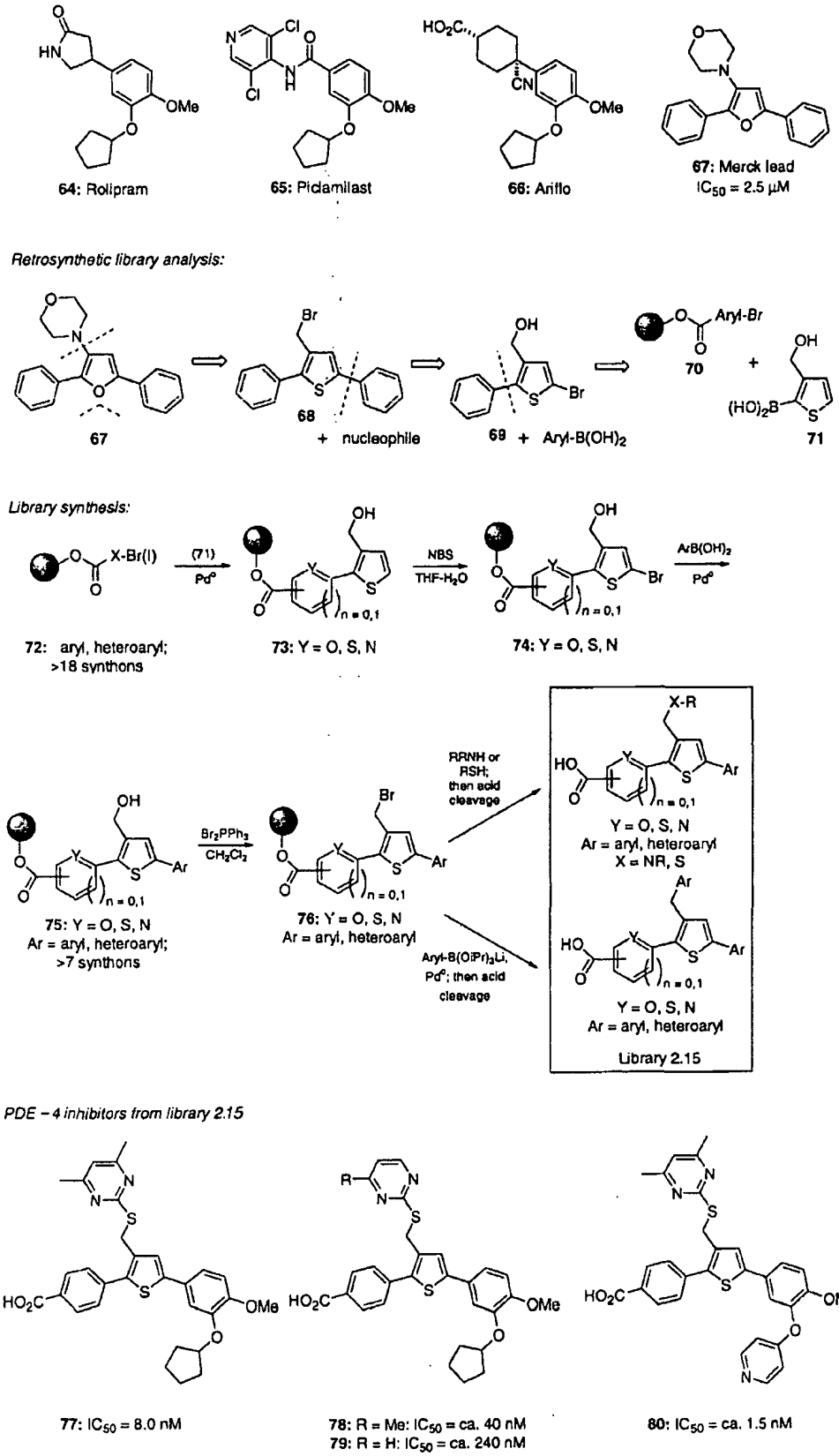


Figure 18. Merck's PDE-4 optimization library 2.15.[93]

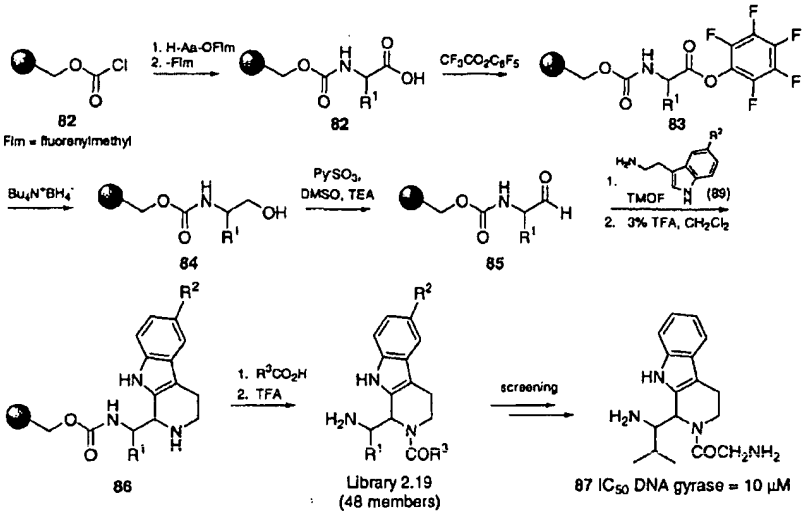


Figure 19. Tetrahydro-β-carboline library yielding DNA gyrase inhibitors.[250]

petitive against ATP-Mg, K_i = 17 μM). Preferred ring substituents include the *p*-hydroxyphenyl and *p*-hydroxybenzyl groups. The small nonpeptide, nonnucleotide class of compounds is structurally unique among known kinase inhibitors. Lead **42** is an inhibitor of colony formation of HT-29 colon adenocarcinoma cells that are dependent on Src activity.

A four-component Ugi condensation was used to create three libraries (2.4a–c) containing potential phosphatase inhibitors (Figure 15).[14] These libraries incorporated a selection of known phosphate mimics as either the aldehyde or acid Ugi reaction partners. Libraries were screened against cell cycle phosphatase Cdc25, an oncology target. A number of structures (e.g., **44**–**46**) were found to be active. Potencies of resynthesized compounds ranged from 0.7 to 35 μM.

Protein farnesyltransferase (FTase) is responsible for the farnesylation of oncogenic Ras proteins, a posttranslational modification required for membrane association and signal transduction. Inhibitors of FTase block mitogenic signaling pathway leading to uncontrolled cell division; hence, the enzyme is an attractive target for cancer chemotherapy. Abbott produced two optimization libraries 2.6[8] and 2.7[101] in an effort to enhance the pharmacokinetic properties of their lead **47** (Figure 16). Biaryl **47** is a potent, non-cysteine, inhibitor of FTase (IC_{50} = 0.4 nM) and active in whole cells. In-house studies suggested the pyridinyl ether in **47** is a metabolic liability, presumably through unwanted formation of the pyridine *N*-oxide. Evidence for this was obtained upon replacement of the pyridinyl ring with a furfuryl moiety, which afforded ether **48** having 30% oral bioavailability in the rat, albeit reduction in enzyme affinity. Bromide **49** was an advanced intermediate used for the generation of a library of furanylbiaryls (library 2.6) via solution-phase Suzuki coupling. A number of potent FTase inhibitors were found in the library. In particular, the 5-(4-chlorophenyl)furfuryl ether **50** restored enzyme and cellular potency and was found to have reasonable pharmacokinetic properties.

In library 2.7, benzylamines were explored as replacements of the pyridinyl ether in **47**.[101] Resin-bound aldehyde **51** was subjected to reductive amination with a host of amines. Inhibitor **52**, derived from cyclohexylalaninol, demonstrated nanomolar activity (IC_{50} = 8 nM). Modification of the biaryl to append an *o*-methyl substituent and replacing the hydroxyl with a thioethyl group furnished inhibitor **54** having high in vitro (IC_{50} = 0.2 nM) and cellular activity (EC_{50} = 4.4 nM). Compound **54** was also active in vivo.

Erm (erythromycin-resistance) family of methyltransferases catalyzes the mono- and dimethylation of the N6-amino group in adenine using S-adenosylmethionine (AdoMet) as a methyl source. This action results in base-specific 23S ribosomal RNA methylation, preventing the binding of certain macrolide antibiotics, and is the mechanism by which pathogenic bacteria may become resistant to these antibiotics. Studies have shown that inhibitors of Erm methyltransferase in combination with a broad-spectrum macrolide antibiotic may be useful in treating resistant bacteria. Using SAR by NMR, triazine **55** (and several other classes of small molecules) was identified as a weak inhibitor of ErmAM methyltransferase (Figure 17).[89] The compound caused chemical shift changes in Erm protein-bound *S*-adenosyl-L-homocysteine (**63**; SAH), and its binding was competitive with this naturally occurring Erm inhibitor. In-house analogues of **55** showed that the activity of this class could be modulated by varying ring substituents (e.g., **56**–**58**), leading to the synthesis of the piperidinylaminotriazine **59** (K_i = 75 μM). Keeping the amino and piperidinyl substituents in **59** constant, library 2.8a of 232 members was created. Evaluation of the library revealed the 2-aminoindanyl as a particularly effective synthon, yielding an ErmAM inhibitor **60**: K_i = 8 μM. The corresponding 1-aminoindanyl congener was 10-fold less active. Library 2.8b further explored the SAR of the class wherein the amino and indanyl substituents were held constant while varying the piperidinyl group. Thislibrary led to a further 2-fold increase in potency; the anilino group was preferred over piperidine (**60** → **62**). Further NMR and X-ray crystallographic studies indicated that the anilino group in **62** partially fills the space occupied by the ribose ring of SAH (**63**), while the amino acid portion

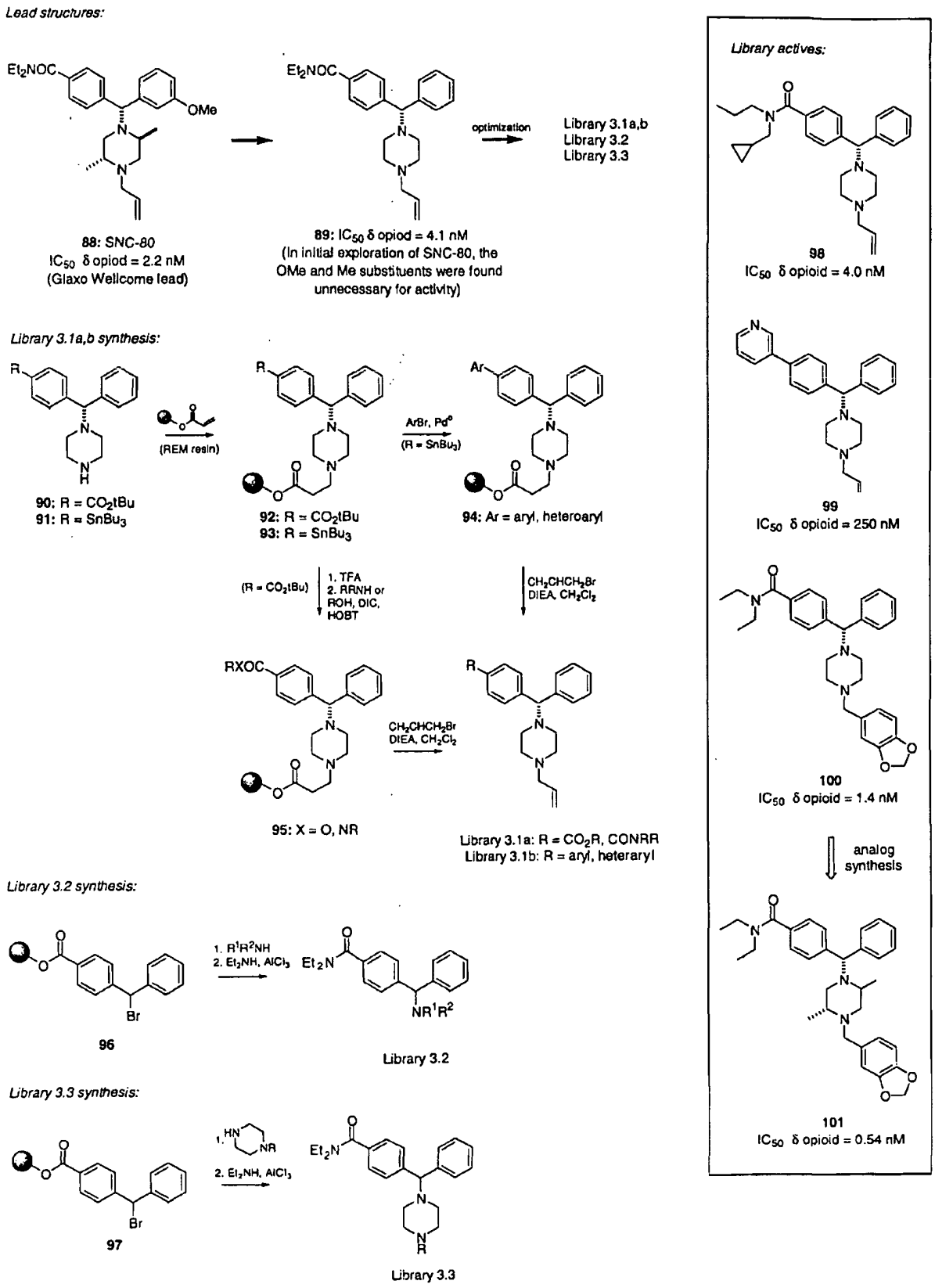


Figure 20. Delta opioid ligands from Organon's libraries 3.1a,b–3.3.[11,55]

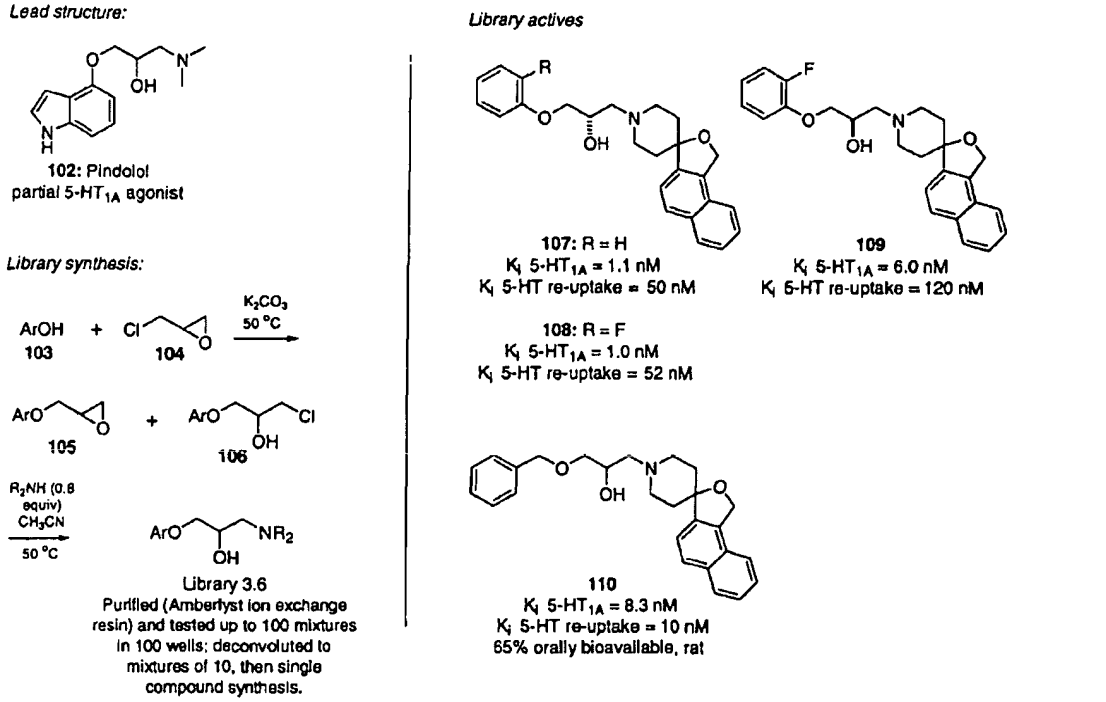


Figure 21. Merck's library of 3-aryloxy-2-propanolamines.[229]

of SAH is completely unoccupied. This suggests that additional gains in potency may be achieved by further structural modifications of **62**, engaging unoccupied binding sites in ErmAM.

The solid-phase synthesis of highly substituted thiophene derivatives and their activity against the cyclic nucleotide phosphodiesterase-4 (PDE-4) enzyme were described by researchers at Merck Frosst (Figure 18).[93] PDE-4 is a member of a broad class of hydrolases and is primarily responsible for the hydrolysis of cAMP in inflammatory and immune cells. Rolipram **64**, piclamilast **65**, and ariflo **66** are examples of PDE-4 inhibitors currently in development for the treatment of depression, rheumatoid arthritis, and asthma, respectively. Library 2.15 was based on the lead structure **67**, a trisubstituted furan, presumably identified through screening an internal compound collection, possessing an IC_{50} = 2.5 μM against PDE-4. Initial SAR revealed that furan and thiophene cores were interchangeable providing that the 2- and 5-aryl rings remained intact. In contemplating a library design, the biaryl rings were thought to be introduced by Suzuki-type couplings. A bromomethyl group was fixed at position C(3) to facilitate the introduction of a broad range of substituents at this position via nucleophilic substitution. For the actual solid-phase synthesis, bromo- and iodo-substituted aromatic carboxylic acids were first attached to Wang resin to give **72**. Suzuki coupling to readily available boronic acid **71** furnished hydroxymethylthiophene intermediate **73**. Bromination of the resin-bound **73** using 2 equiv of NBS in THF containing 2% water occurred in high yield *without* compromising the linker. Bromide **74** in turn was subjected to a second Suzuki coupling with a host of aryl and heteroaryl boronic acids to give the 2,5-biaryl-3-hydroxymethylthiophenes **75**. Conversion of the hydroxy group to the corresponding bromide was accomplished using bromotriphenylphosphine bromide in methylene chloride (**75** → **76**). Bromomethyl intermediate **76** was treated with a range of nitrogen and sulfur nucleophiles, affording library 2.15. Alternatively, carbon−carbon bond formation via Pd-catalyzed coupling of **76** with lithoaryltriisopropylboronates led to the direct exchange of the bromine atom for an aryl ring. One of the more potent PDE-4 inhibitors identified from the library was **77**, IC_{50} = 8.0 nM. This inhibitor contained the 3-cyclopentyloxy-4-methoxyphenyl ring at C(5), a substituent shared by known PDE-4 inhibitors **64-66**.

DNA gyrase inhibitors were identified from a library of tetrahydro-β-carbolines (library 2.19, Figure 19).[250] The key intermediate for the library was the resin-bound amino acid aldehyde **85** prepared by sequentially attaching amino acid fluorenylmethyl esters to chlorocarbonate resin, followed by deprotection, reduction of the corresponding activated pentafluorophenyl esters with tetrabutylammonium borohydride, and oxidation with sulfur trioxide-pyridine complex (**82** → **83** → **84**). Pictet−Spengler reaction of **85** with a series of tryptamines and then derivatization of the resulting secondary amino function of the tetrahydro-β-carboline furnished library 2.19.

Libraries Yielding G-Protein Coupled Receptor Agonists and Antagonists

Entries in Table 3 refer to those libraries that have yielded agents with binding affinity toward G-protein coupled receptors (GPCRs). Within the table are libraries active against opioid receptors (libraries 3.1−3.5), serotonin receptors (libraries 3.6 and 3.7), somatostatin receptors (libraries 3.8−3.9), and assorted miscellaneous receptors (libraries 3.10−3.15).

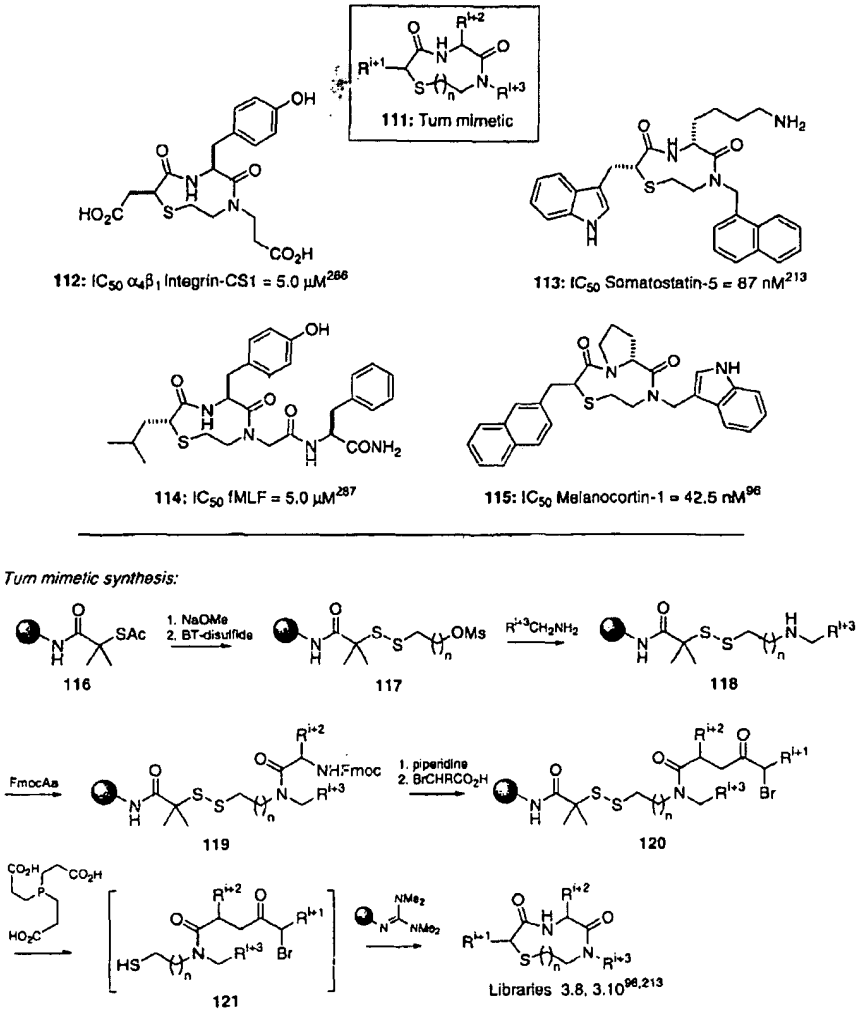


Figure 22. Biological activities of Ellman's turn mimetic library.[213]

Several focused libraries of δ opioid ligands based on the Glaxo Wellcome lead SNC-80 **88** were synthesized at Organon (Figure 20).[11,55] Initial SAR studies indicated that the aryl methoxy and piperazinyl methyl groups were not critical for δ opioid affinity, but the *N*,*N*-diethylcarboxamide was an essential structural feature. This is represented by structure **89**: δ opioid IC_{50} = 4.1 nM, and >1000-fold selective versus the μ and κ opioid receptors. In an effort to further explore the SAR, four optimization libraries were prepared. Libraries 3.1a and 3.1b relied on REM resin methodology to strategically target the carboxamide group for modification. In this chemistry, piperazines **90** and **91** were coupled to REM resin to give ester **92** and stannane **93**, respectively. Selective deprotection of the *tert*-butyl ester group with TFA furnished the corresponding acid, which in turn was converted to either an ester or amide (**92** → **95**). Stannane **93** was subjected to Stille coupling to 10 aryl and heteroaryl bromides (**93** → **94**). Release of library compounds was achieved after quaternization of resins **94** and **95** with allyl bromide and Hofmann elimination (Hunig's base, 18 h, 20 °C). No significant improvement in activity was observed.

In complementary optimization libraries 3.2 and 3.3, piperazine replacements (cyclic diamines) and *N*-substituted piperazines were investigated (Figure 20).[11] In these libraries the diethylcarboxamide group was retained. None of the cyclic diamines were as active as piperazine, but a 4-fold improvement in binding affinity was observed when piperonyl was substituted for allyl; **100**: IC_{50} = 1.4 nM. Reintroducing the dimethyl groups and piperazine stereochemistry as per SNC-80, gave **101** with subnanomolar potency against the δ opioid receptor.

A solution-phase synthesis was developed for the preparation of 3-aryloxy-2-propanolamine libraries (library 3.6, Figure 21).[229] Specific interest in this class of compound stems from an interest in identifying dual affinity 5-HT_{1A} and 5-HT re-uptake ligands as potential antidepressants with improved side effects. The library design focused on modifying pindolol **102** (partial 5-HT_{1A} agonist). A diverse set of amine and phenol synthons were utilized in the library. These were obtained from commercial sources and in-house "privileged structures", as well as selections based on amine fragments from serotonin re-uptake blockers and substituted phenols from 5-HT_{1A} ligands. Binding data were first

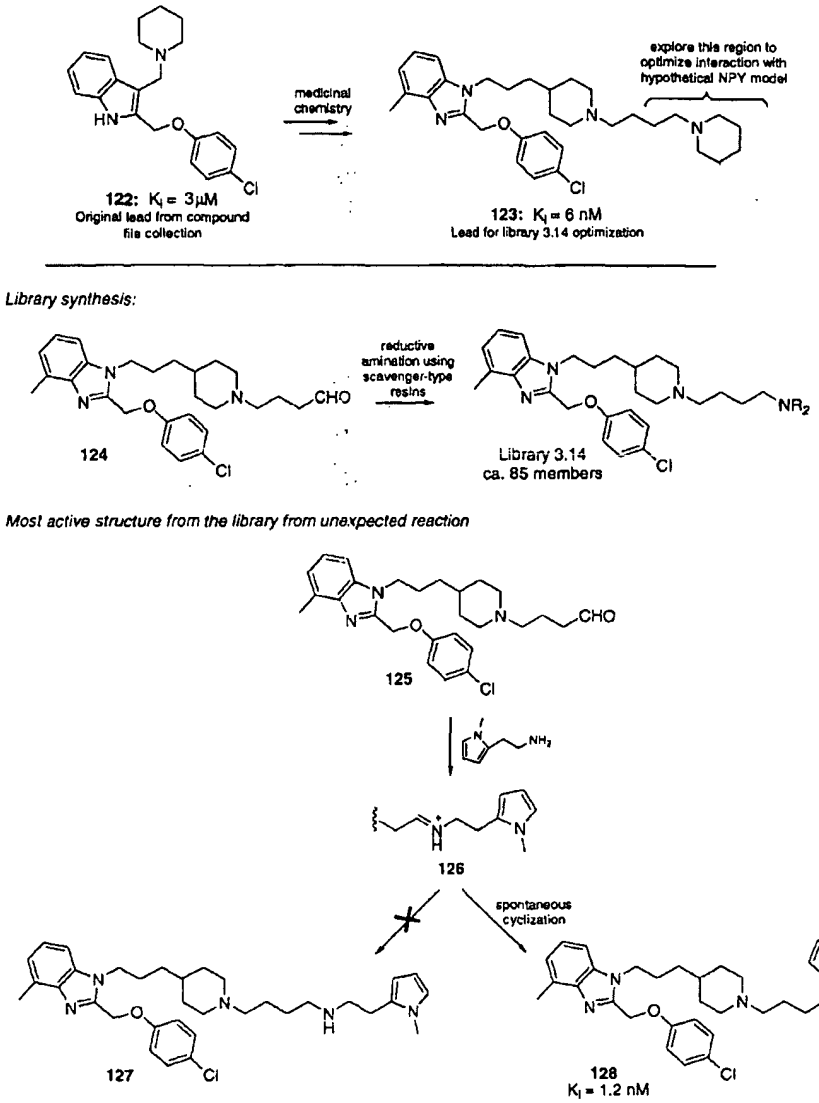


Figure 23. Neuropeptide Y-1 antagonists from library 3.14.[205]

obtained on purified mixtures containing up to 100 compounds per well, then deconvoluted to yield single compounds. Several potent 5-HT_{1A} ligands were identified. The simple substituted phenols were found to be superior to the indole in pindolol **102**. The spirocyclic amine found in **107**–**110** was the only amine to give consistent levels of dual activity (binding at serotonin re-uptake receptors and 5-HT_{1A} receptors). Compound **110** demonstrated nearly full agonism at 5-HT_{1A} and potent re-uptake blocking properties. Compound **110** was found to be 65% orally bioavailable in the rat (3 mg/kg) possesing a $t_{1/2}$ = 3.0 h.

Ellman published a full report on the synthesis of a turn mimetic library (libraries 3.8 and 3.10; Figure 22).[96,213] The synthesis takes advantage of a facile intramolecular-cyclative thiol S_N2 displacement, simultaneously cleaving material from resin and creating the penultimate 9- and 10-member rings. Preliminary reports of this chemistry have appeared in the literature as well as multiple biological activities associated with this interesting class of medium-sized heterocycles. Turn mimetics have shown activity as integrin antagonists,[286] human neutrophile receptor (fMLF) inhibitors,[287] and selective agonists against somatostatin-5 [213] and melanocortin-1.[96]

A full disclosure of the library synthesis (library 3.9) and screening of Merck's selective somatostatin receptor agonists was published this past year.[158, 288]

Neuropeptide Y, found in both the peripheral and central nervous systems, is believed to be involved in the regulation of feeding, energy metabolism, vascular tone, learning and memory, and the release of pituitary hormones. To date, six receptors of this family have been characterized pharmacologically. Several antagonists of the NPY-1 receptor have been reported in the literature. One class of compounds discovered at Lilly is the benzimidazoles, represented by structure **123** (Figure 23).[205] The potent NPY-1 antagonist was obtained following extensive medicinal chemical optimization, starting from the 3 μM in-house screening hit **122**. Using the combined applications of computational chemistry

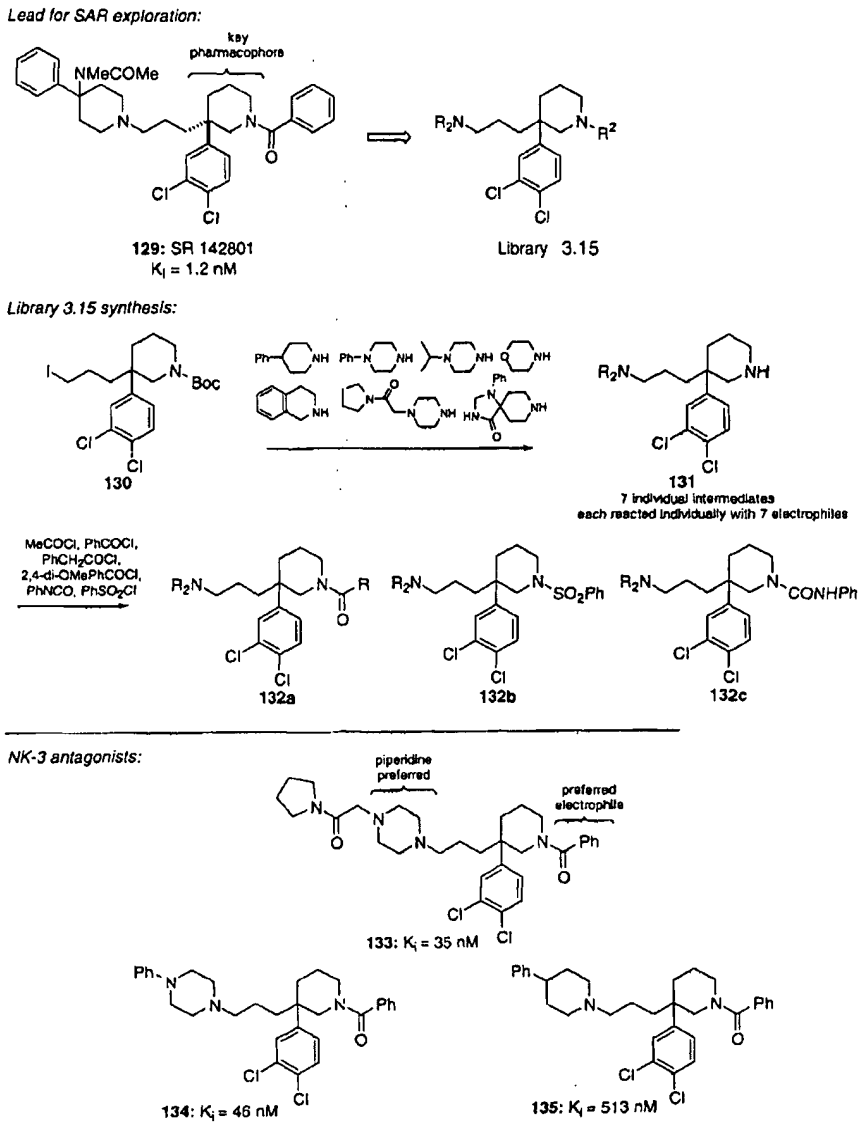


Figure 24. Human neurokinin-3 receptor antagonists.[188]

and parallel solid-phase synthesis, further optimization of **123** was undertaken. Computer modeling suggested that the interactions between the distal piperidine group and salient residues in the operative NPY-1 model may not be optimal for high affinity binding. With this hypothesis in mind, library 3.14 was constructed to explore alternative amines. Chemistry was carried out via the reductive amination using resin-bound aldehyde **124** and ca. 100 amines. Amines were selected by initially searching the ACD database of commercially available primary amines (6642 matches). The "master list" was reduced in a first round by discarding amines containing carboxylic acids and MW >250 (1636 matches) and in a second round by similarity clustering (577 matches). A final list of amine synthons was generated by human selection to <100 amines. Some 85−90 compounds were prepared in the library and each evaluated against NPY-1. Only one compound appeared more active than **123**, and that was a compound obtained from the reductive amination of aldehyde **124** with *N*-methyl-2-aminoethylpyrrole. However, the structure of the expected product **127** was inconsistent with its spectroscopic data. It turned out that, in this particular case, the intermediate imine **126** undergoes a spontaneous Pictet−Spengler cyclization affording tetrahydro-5-aza-indole **128**. This is the *second* solid-phase synthesis example where the occurrence of an unanticipated side reaction yielded a biologically active agent.[289]

An interesting application of combinatorial library synthesis is in the rapid evaluation of "competitor compounds". It is not uncommon in the pharmaceutical industry to have multiple companies simultaneously pursue drug discovery programs focused on an identical molecular target in the race to be first to market with a breakthrough drug. For this reason, when a competitor publishes the structure and biological activity of a "hot target", other research groups will prepare this compound and use it as a benchmark against their own series. In many instances however, much of the

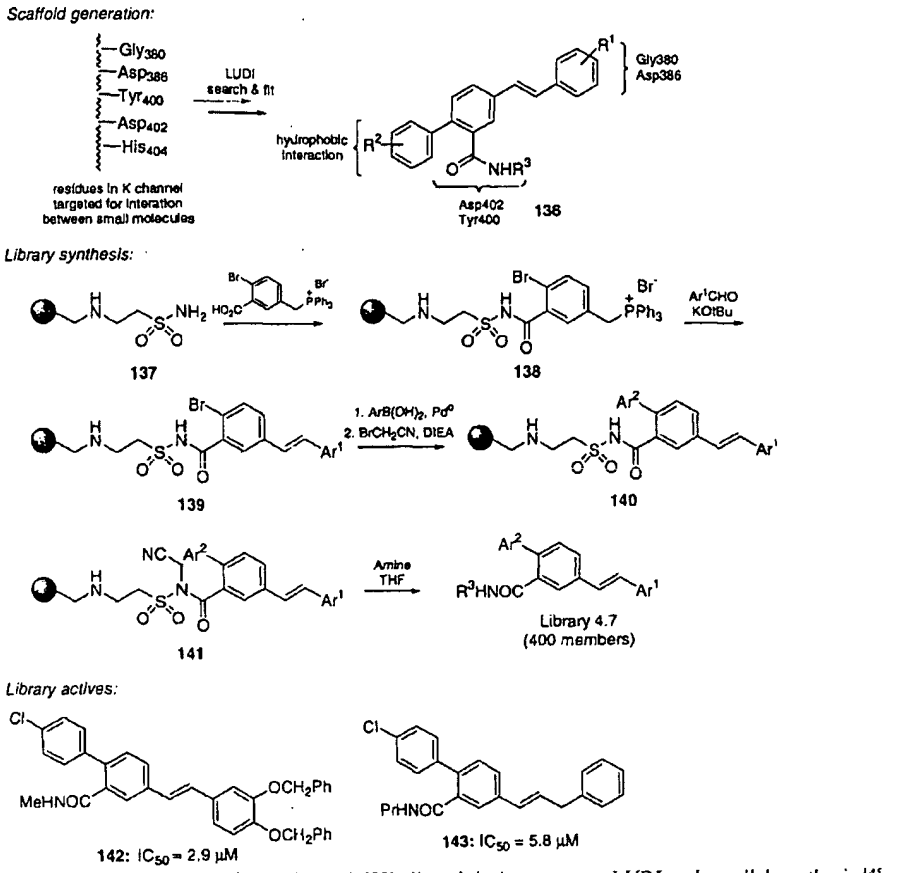


Figure 25. Potassium channel blockers obtained from Biosym/MSI's ligand design program LUDI and parallel synthesis.[145]

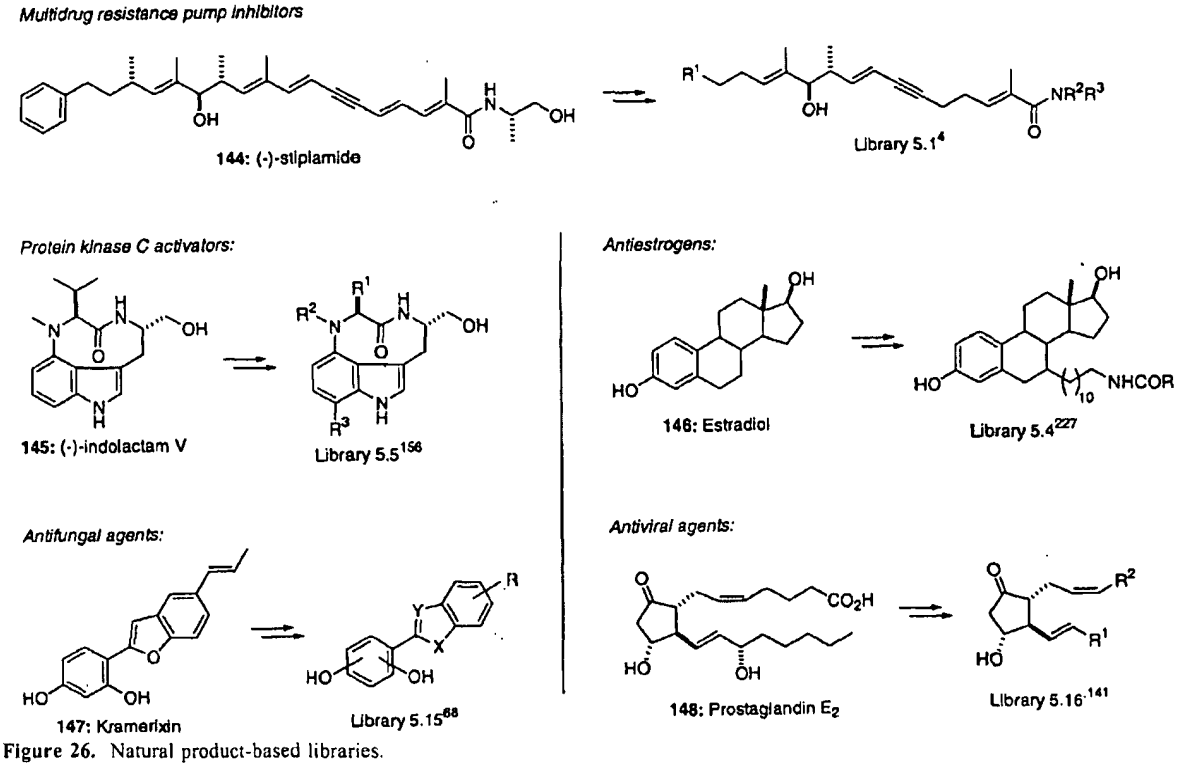


Figure 26. Natural product-based libraries.

SAR around the structure is unpublished and kept secret, although this information can be invaluable in enhancing one's own lead series. Library 3.15 is an optimization library synthesized at SmithKline Beecham and designed to explore the SAR requirements of the 3,4-diclorophenylpiperidine class of NK-3 receptor antagonists (Figure 24).[188] This class of agent was first reported by Sanofi (e.g., SR 142801: **129**), but SAR data was virtually nonexistent in the literature. Using a combinatorial chemical approach, SmithKline was able to rapidly generate analogues of interest. This was carrried out synthetically by sequential reaction of the bifunctional derivative **130**, derived from the key (3,4-dichlorophenyl)-3-propylpiperidine pharmacophore, with amines and electrophiles (library 3.15: **132a–c**).[188]

Libraries Yielding Non-GPCR Ligands

Libraries yielding active structures against non-GPCR molecular targets are delineated in Table 4. Table 4 is subdivided into integrin receptors (libraries 4.1–4.5), ion channels (libraries 4.6 and 4.7), and miscellaneous targets (libraries 4.8–4.12).

Three of the five libraries describing integrin antagonists were direct takeoffs of the well-known -Arg-Gly-Asp- binding motif linking basic guanidyl and carboxylic acid residues through an optimal spacer. Library 4.1 utilizes an azapeptide-type spacer[77] while isoxazole linkers were utilized by DuPont (libraries 4.3 and 4.4).[190] A new binding motif, D-Pro-D-Tyr-D-Leu-, identified in Selectide's library 4.2 is of interest as it is a neutral ligand,[223] although its affinity is rather weak (14 μM) compared to classical charged ligands possessing nanomolar affinity. Optimization library 4.5 was part of a broad-based medicinal chemistry effort to identifiy potent integrin antagonists incorporating piperidine as a surrogate for the guanidyl residue.

An interesting new series of phenyl substituted stilbenes as voltage gated potassium channel blockers were described by Lew and Chamberlin (library 4.7, Figure 25).[145] The stilbene pharmacophore was computationally designed using LUDI, a Biosym/MSI ligand design program, and predicted to block the potassium channel. A combinatorial library of LUDI hits was generated furnishing μM leads **142** and **143** for further studies.

Libraries of Cytotoxic and Antiinfective Agents

Table 5 contains 17 libraries subdivided into two categories demonstrating cytotoxic activity (libraries 5.1–5.5) and antiinfective activity, including antibacterials (libraries 5.6–5.13), antifugals (libraries 5.14–5.15), and antivirals (libraries 5.16 and 5.17). One reoccurring theme in this set of entries is the use of natural products as templates or starting points for library design. Examples of this include (−)-stipiamide-based library 5.1,[4] estradiol-based library 5.4,[227] (−)-indolactam V-based library 5.5,[156] kramerixin-based library 5.15,[68] and the prostanoid-based library 5.16 (Figure 26).[141]

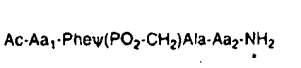
Isis described a series of libraries using a technique of "simultaneous addition of fuctionality" in which chemically reactive polyhalogenated heterocycles are treated with excess nucleophiles to create libraries possessing antibacterial activity (libraries 5.11–5.13).[119] No specific compounds were identified from the libraries. Polyhalogenated heteroaromatics as well as the corresponding reactive fluoronitroaromatics have been used extensively over the past several years in the synthesis of biologically active libraries and other library constructs of medicinal interest (Figures 6 and 7).

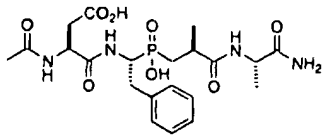
Acknowledgment. Many thanks to Ms. Karen Rivera for her invaluable assistance in the preparation of this manuscript and for her expertise and perseverance in chemical structure drawing.

Table 1. Chemical Libraries Targeted for Proteases[a]

Metallo-proteases

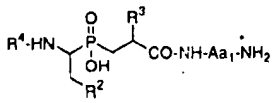
Library: 1.1
Name: Phosphinic peptide
Size: 400 members
Affiliation: Dive, V.; *et al.* [64]
Note: Twenty peptide mixtures each containing a single Aa_1 with a mixture of 20 Aa_2.

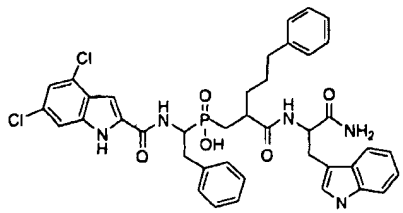




Enzyme: Somatic angiotensin converting enzyme (ACE)
Activity: K_i = 12 nM, ACE (N-domain); K_i = 25 μM, ACE (C-domain)

Library: 1.2
Name: Phosphinic tripeptide
Size: Not defined
Affiliation: Dive, V.; *et al.* [230]
Note: Several libraries prepared.

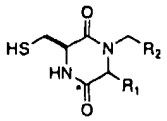


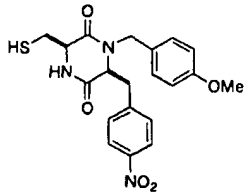


Enzyme: MMP-11 (stromelysin-3)
Activity: K_i = 0.9 nM (K_i = 24 nM, MMP-2; K_i = 7 nM, MMP-9; K_i = 32 nM, MMP-14; K_i = 36 nM, MMP-1; K_i = 117 nM, MMP-7; K_i = 5 nM, MMP-8)

Table 1. (Continued)

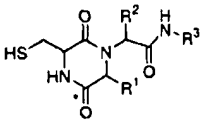
Library: 1.3
Name: Diketopiperazine
Size: 1225 members
Affiliation: Affymax Res. Inst. [221]

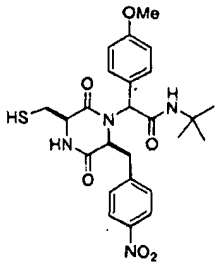




Enzyme: Collagenase-1
Activity: IC_{50} = 47 nM (IC_{50} = 1200 nM, gelatinase-B; >4000 nM, stromelysin)

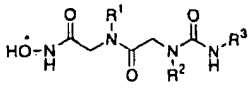
Library: 1.4
Name: Diketopiperazine
Size: ca. 700-900 members
Affiliation: Affymax Res. Inst. [221]
Note: Three-component Ugi condensation.

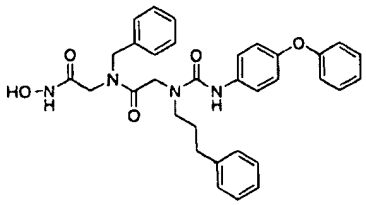




Enzyme: Collagenase-1
IC_{50} = 21 nM (IC_{50} = 1300 nM, gelatinase-B)

Library: 1.5
Name: Urea-based hydroxymate
Size: Not defined
Affiliation: Lauhon, C. T.; *et al.* [247]

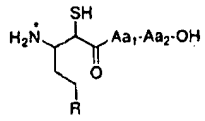


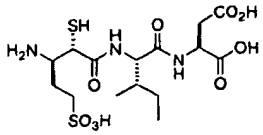


Enzyme: Gelatinase (MMP-2)
Activity: IC_{50} = 300 nM

Aspartic acid proteases

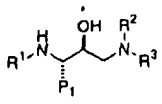
Library: 1.6
Name: Pseudotripeptide
Size: 380 members
Affiliation: Roques, B. P.; *et al.* [59]
Note: Two positional scanning libraries.

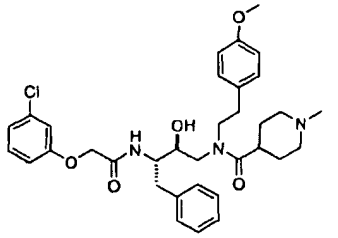




Enzyme: Aminopeptidase A
Activity: K_i = 3.2 nM

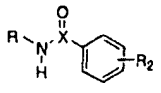
Library: 1.7
Name: Hydroxyethylamine
Size: ca. 170 members
Affiliation: Ellman, J. A.; *et al.* [95]
Note: Six iterative libraries for lead optimization.

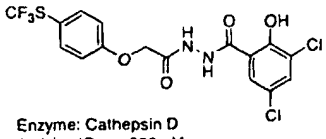




Enzyme: Plasmepsin II (*P. falciparum*)
Activity: K_i = 2 nM (K_i = 9.8 nM, cathepsin D)

Library: 1.8
Name: Amide derivative
Size: 300 members
Affiliation: Bayer Corp. [47]
Note: Solution-phase synthesis using polymer bound reagents.



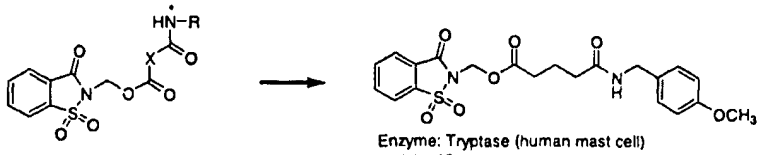


Enzyme: Cathepsin D
Activity: IC_{50} = 320 nM

Table 1. (Continued)

Serine proteases

Library: 1.9
Name: Benzisothiazolone
Size: 60 members
Affiliation: Bristol-Myers Squibb [243]
Note: Encoded split-pool library using R_f tags.



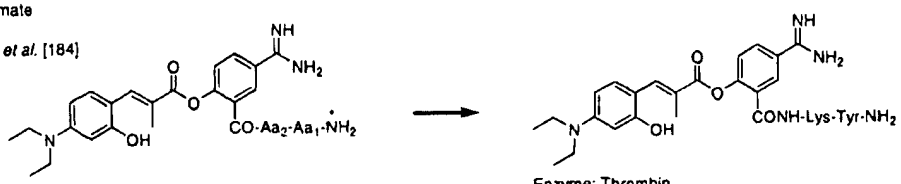
Enzyme: Tryptase (human mast cell)
Activity: IC_{50} = 0.23 μM

Library: 1.10
Name: Peptide ketothiazole
Size: ca. 150 members
Affiliation: Organon Labs [3]



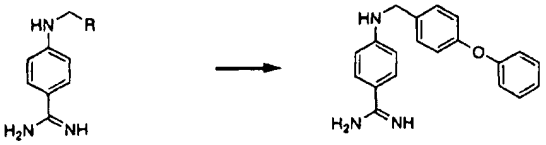
Enzyme: Factor Xa
Activity: IC_{50} = 0.43 μM
(IC_{50} = 4.8 μM, thrombin)

Library: 1.11
Name: *o*-Hydroxycinnamate
Size: 112 members
Affiliation: Porter, N. A.; *et al.* [184]



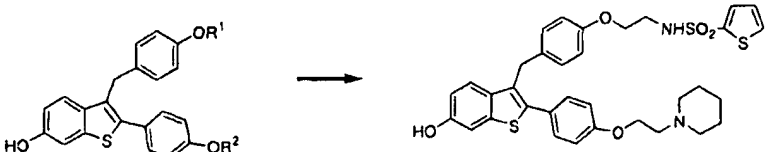
Enzyme: Thrombin
Activity: Photo-reversible inactivation

Library: 1.12
Name: Phenylamidine
Size: 10 members
Affiliation: Hoffmann-La Roche [22]
Note: Combinatorial docking procedure led to selection of library compounds.

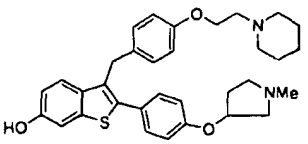


Enzyme: Thrombin
Activity: K_i = 9.5 nM
(K_i = 520 nM, trypsin)

Library: 1.13
Name: Benzothiophene
Size: 346 members
Affiliation: Sphinx Pharm. [114]
Note: Solid-phase Mitsunobu chemistry and parallel purification.

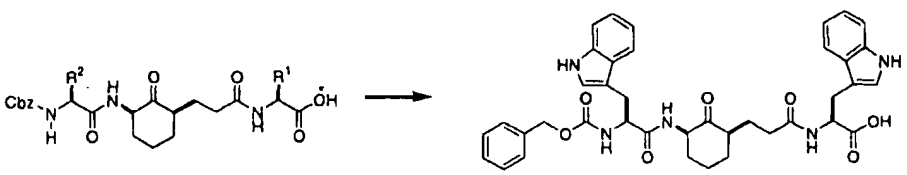


Enzyme: Thrombin (human)
Activity: K_{ass} = 1μM (0.02 μM, Factor Xa)



Enzyme: Factor Xa (human)
Activity: K_{ass} = 5.0 nM (2.3 μM, thrombin)

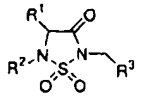
Library: 1.14
Name: Cyclohexanone peptide
Size: 400 members
Affiliation: Seto, C. T.; *et al.* [2]

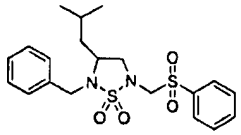


Enzyme: Plasmin
Activity: K_i = 5 μM

Table 1. (Continued)

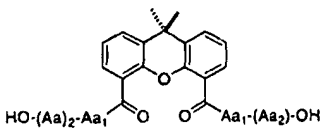
Library: 1.15
Name: 1,2,5-Thiadiazolidin-3-one
Size: ca. 12 members
Affiliation: Groutas, W. C.; *et al.* [128]
Note: Solution-phase synthesis.

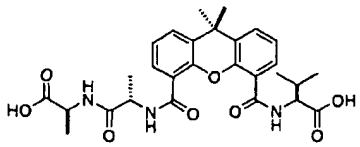




Enzyme: Elastase (human leukocyte)
Activity: k_{inact}/K_i = 95,000 $M^{-1}s^{-1}$

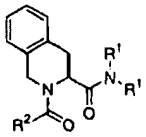
Library: 1.16
Name: Disubstituted xanthenes
Size: ca. 12 members
Affiliation: Pryor, K. E.; *et al.* [186]
Note: Two-phase colorimetry based screen for inhibition.

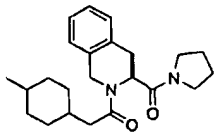




Enzyme: (human leukocyte) Elastase
Activity: K_i = 86 μM

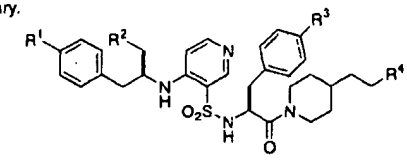
Library: 1.17
Name: Tetrahydroisoquinolin
Size: 2560 members
Affiliation: Sergheraert, C.; *et al.* [232]
Note: Solution-phase synthesis.

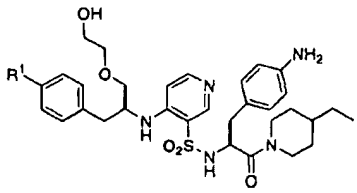




Enzyme: Prolyl endopeptidase (*Trypanosoma cruzi*)
Activity: IC_{50} = 9.0 nM

Library: 1.18
Name: Sulfonamides
Size: 198 members
Affiliation: Novartis [252]
Note: Optimization library.

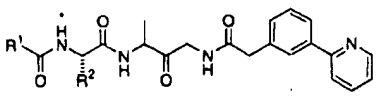


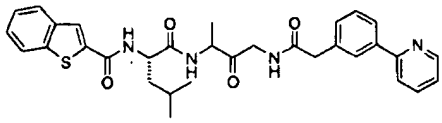


Enzyme: Thrombin
Activity: K_i = 147 nM (55% oral bioavailability (30 mg/kg, p.o.) in rat; $t_{1/2}$ = 120-180 min; C_{max} = 3.36 mg.mL^{-1}

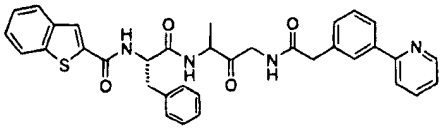
Cysteine proteases

Library: 1.19
Name: Acylaminobutanone
Size: 18 members
Affiliation: SmithKline Beecham [239]
Note: R_1 encoding.





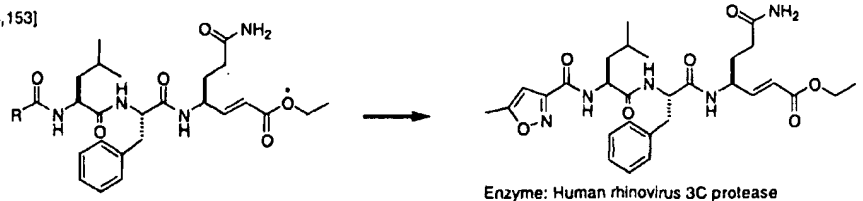
Enzyme: Cathepsin K
Activity: $K_{i,app}$ = 1.3 nM ($K_{i,app}$ = 90 nM, cathepsin L; $K_{i,app}$ = >1000 nM cathepsin B)



Enzyme: Cathepsin L
Activity: $K_{i,app}$ = 18 nM ($K_{i,app}$ = 16 nM cathespin K; $K_{i,app}$ = >1000 nM, cathepsin B)

Table 1. (Continued)

Library: 1.20
Name: Tripeptide Michael acceptor
Size: ca. 500 members
Affiliation: Agouron Pharm. [66,153]



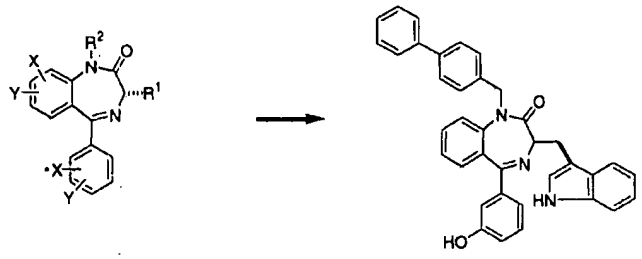
Enzyme: Human rhinovirus 3C protease
Activity: $K_{obs}/I = 260{,}000\ M^{-1}s^{-1}$

[a] Asterisk (*) indicates point of attachment to the resin.

Table 2. Chemical Libraries Targeted for Nonproteolytic Enzymes[a]

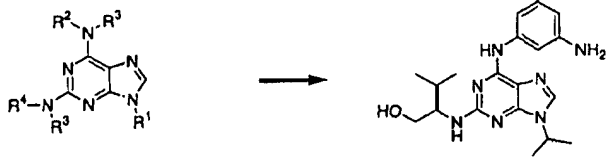
Kinases and phosphatases

Library: 2.1
Name: 1,4-Benzodiazepine
Size: 1680 members
Affiliation: Ramdas, L.; *et al.* [187]



Enzyme: Src protein tyrosine kinase
Activity: $IC_{50} = 35\ \mu M$

Library: 2.2
Name: Substituted purine
Size: Not defined
Affiliation: Chang, Y. T.; *et al.* [44]
Note: Multiple solution- and solid-phase libraries.



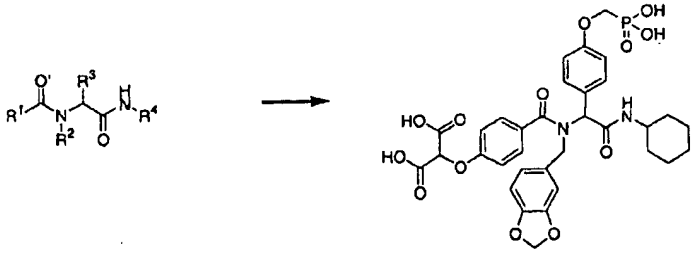
Enzyme: Cyclin-dependent kinase 2 (CDK2)
Activity: $IC_{50} = 33$ nM ($IC_{50} = 28$ nM, CDK1)

Library: 2.3
Name: Octapeptide
Size: ca. 110,000 members
Affiliation: Watterson, D. M.; *et al.* [150]
Note: Positional scanning.

H-Arg-Lys-Lys-Aa_1-Aa_2-Aa_3-Arg-Arg-Lys-NH_2 → H-Arg-Lys-Lys-Tyr-Lys-Tyr-Arg-Arg-Lys-NH_2

Enzyme: Smooth muscle myosin light chain kinase (chicken)
Activity: $IC_{50} = 50$ nM (>40,000-fold selective versus calmodulin-regulated protein kinase)

Library: 2.4a-c
Name: Amino acid amide
Size: 4320 total members
Affiliation: Mitotix, Inc. [14]
Note: Three libraries based on Ugi reaction with phosphate surrogates.

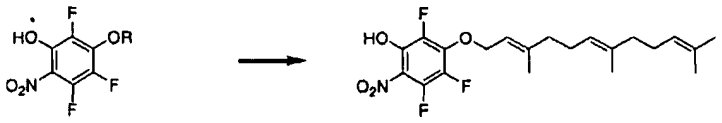


Enzyme: Cdc25 phosphatase
Activity: $IC_{50} = 0.7\ \mu M$
($IC_{50} = 82\ \mu M$, tyrosine phosphatase PTP1B)

Table 2. (Continued)

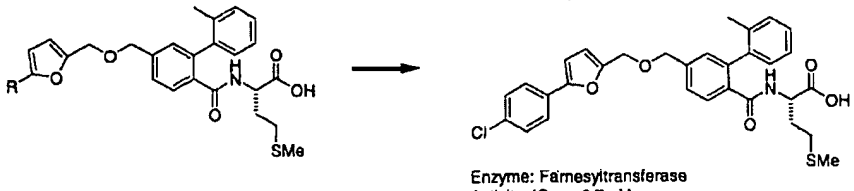
Transferases

Library: 2.5
Name: Trifluoronitrophenol
Size: 12 members
Affiliation: Hardcastle, I. R.; *et al.* [9]



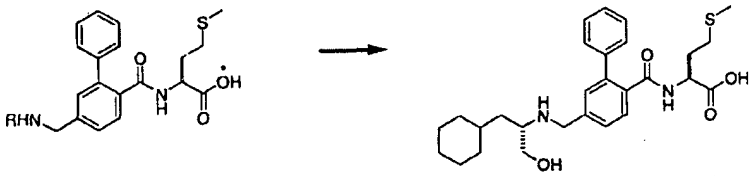
Enzyme: Farnesyltransferase (rat)
Activity: IC_{50} = 6.3 μM
(IC_{50} = 12.5 μM, geranylgeranyl protein transferase-I)

Library: 2.6
Name: Methionine biaryl
Size: 31 members
Affiliation: Abbott [8]
Note: Solution-phase optimization library.



Enzyme: Farnesyltransferase
Activity: IC_{50} = 0.7 nM
(>10,000-fold selective vs. geranylgeranyl protein transferase-1; 21% orally bioavailable, dog)

Library: 2.7
Name: Biphenyl methionine
Size: Not defined
Affiliation: Abbott [101]



Enzyme: Farnesyltransferase (FTase)
Activity: IC_{50} = 8.0 nM

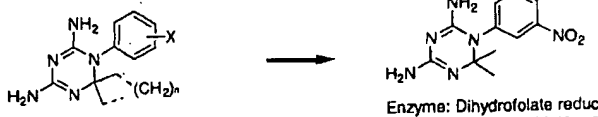
Library: 2.8a,b
Name: Triazine
Size: 643 members
Affiliation: Abbott [89]



Target: Erm methyltransferase
Activity: IC_{50} = 4 μM

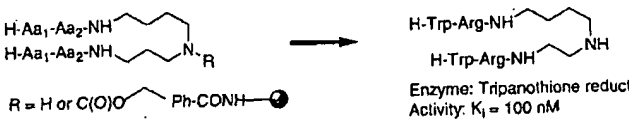
Reductases and dehydratases

Library: 2.9
Name: Dihydrophenyl triazine
Size: 64 members
Affiliation: Chui, W.-K.; *et al.* [139]
Note: Three-component condensation using anilines, ketones, and cyanoguanidine.



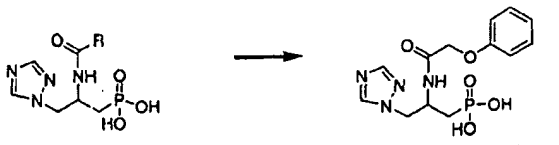
Enzyme: Dihydrofolate reductase
Activity: IC_{50} = 6.0 nM; K_i = 200 μM

Library: 2.10
Name: Polyamine peptide conjugate
Size: 576 members
Affiliation: Bradley, M.; *et al.* [209]
Note: Three identical libraries produced in solution on PEGA resin, and on Tentagel to compare success of solution- versus on-bead assays.



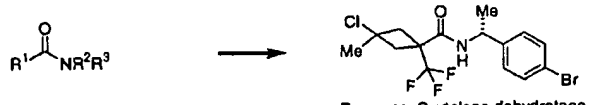
Enzyme: Tripanothione reductase
Activity: K_i = 100 nM

Library: 2.11
Name: β-Carboxamido phosphonate
Size: 15 members
Affiliation: Monsanto Co. [202]
Note: Solution-phase synthesis using solid-phase capture reagents.



Enzymes: Imidazole glycerol phosphate dehydratase (*Cryptococcus neoformans*)
Activity: K_i = 80 nM

Library: 2.12
Name: Amide
Size: 768 members
Affiliation: Du Pont Agricul. [113]
Note: Solution-phase synthesis.

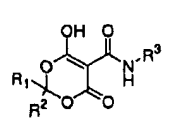


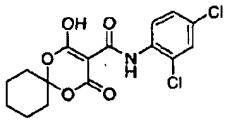
Enzymes: Scytalone dehydratase
Activity: K_i = 0.05 nM

Table 2. (Continued)

Other enzymes

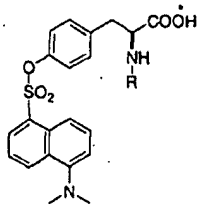
Library: 2.13
Name: 1,3-Dioxane-4,6-dione carboxamide
Size: ca. 113 members
Affiliation: Gelb, M. H.; *et al.* [33]
Note: Solution-phase synthesis.

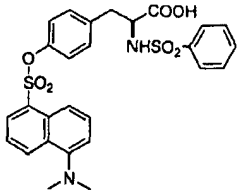




Enzyme: Phospholipase A_2 (human group IIA)
Activity: $X_i(50) = 0.022$

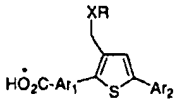
Library: 2.14
Name: Dansyltyrosine
Size: 34 members
Affiliation: Tondi, D.; *et al.* [225]



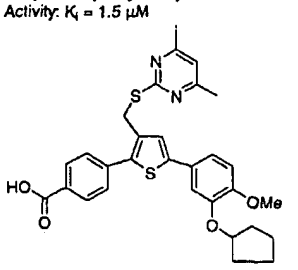


Enzyme: Thymidylate synthase
Activity: $K_i = 1.5$ μM

Library: 2.15
Name: Substituted thiophene
Size: ca. 100 members
Affiliation: Merck Frosst [93]

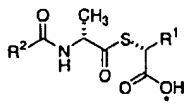


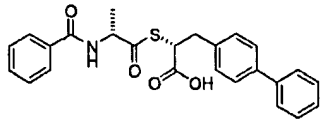
X = NR, S



Enzyme: Phosphodiesterase-4 (PDE-4)
Activity: $IC_{50} = 8.0$ nM

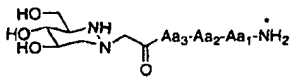
Library: 2.16
Name: Thiodepsipeptide
Size: 38 members
Affiliation: Merck [84]

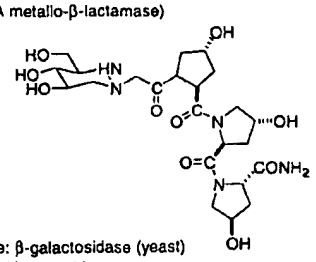




Enzyme: IMP-1 metallo-β-lactamase
Activity: $IC_{50} = 0.4$ nM ($IC_{50} = 180$ nM, CcrA metallo-β-lactamase)

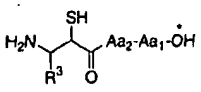
Library: 2.17
Name: 1-Azafagomine peptide
Size: 125 members
Affiliation: Bols, M.; *et al.* [148], [149]

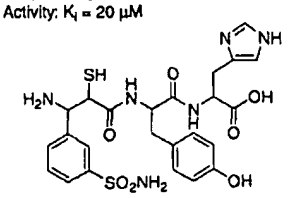




Enzyme: β-galactosidase (yeast)
Activity: $K_i = 20$ μM

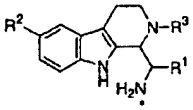
Library: 2.18
Name: β-Aminothiol peptide
Size: ca. 600 members
Affiliation: Roques, B. P.; *et al.* [151]

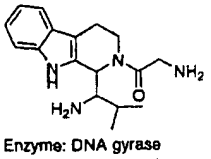




Enzyme: Bacterial protein tetanus toxin (TeNt)
Activity: $K_i = 5.0$ μM

Library: 2.19
Name: Tetrahydro-β-carboline
Size: 48 members
Affiliation: Jung, G.; *et al.* [250]





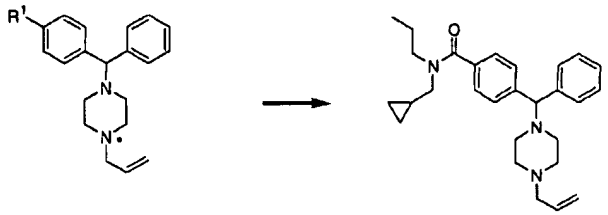
Enzyme: DNA gyrase
Activity: $IC_{50} = 10$ μM

[a] Asterisk (*) indicates point of attachment to the resin.

Table 3. Chemical Libraries Targeted for G-Protein Coupled Receptors[a]

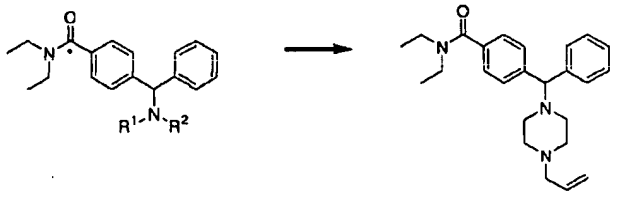
Opioid receptors

Library: 3.1a,b
Name: *N*-Diarylmethylpiperazine
Size: ca. 125 members
Affiliation: Organon Labs. [55]
Note: Solid-phase synthesis using REM resin.



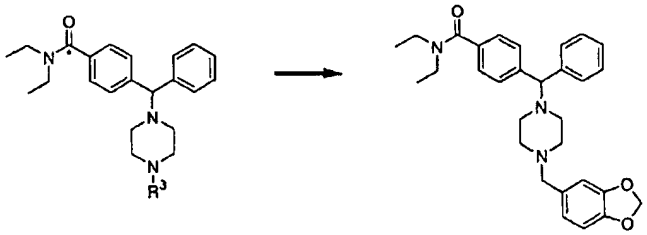
Target: δ opioid (human)
Activity: IC_{50} = 3.0 nM
(IC_{50} = >10,000 nM, μ (rat))

Library: 3.2
Name: Diarylmethylamine
Size: 20 members
Affiliation: Organon Labs. [11]
Note: $AlCl_3$-mediated diethylaminolysis of Wang esters.
See library 3.3.



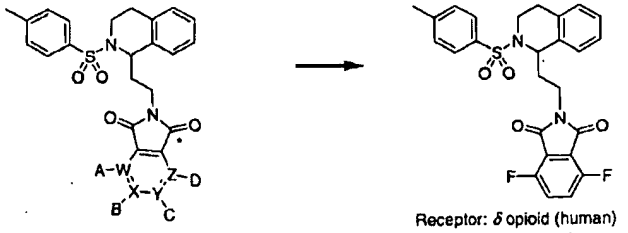
Receptor: δ opioid (human)
Activity: IC_{50} = 8 nM

Library: 3.3
Name: *N*-Diarylmethylpiperazine
Size: 46 members
Affiliation: Organon Labs. [11]
Note: $AlCl_3$-mediated diethylaminolysis of Wang esters.
See library 3.2.



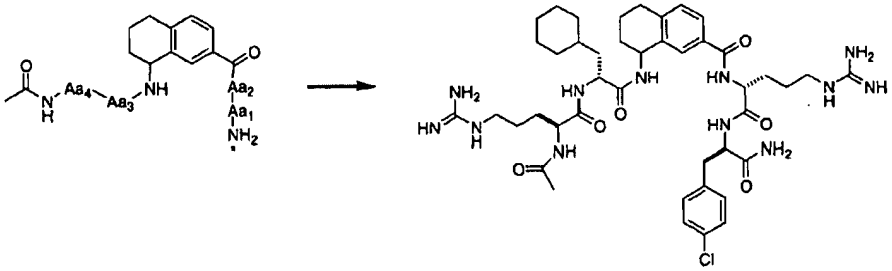
Receptor: δ opioid (human)
Activity: IC_{50} = 1.4 nM
(IC_{50} = >10,000 nM, μ (rat);
IC_{50} = 3900 nM, κ (guinea pig))

Library: 3.4
Name: Cyclic imide
Size: 30 members
Affiliation: Organon Labs. [12]



Receptor: δ opioid (human)
Activity: IC_{50} = 16 nM

Library: 3.5
Name: Pseudopentapeptide
Size: 20,000,000 members
Affiliation: Simonin, F.; *et al.* [13]
Note: Positional scanning at Aa_4.

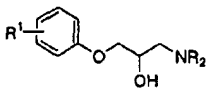


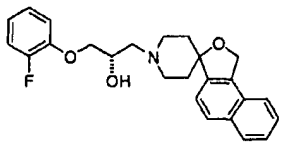
Receptor: Opioid receptor-like 1 (ORL-1, human)
Activity: K_i = 517 nM (K_i = 114 nM, κ-opioid;
K_i = 1300 nM, μ-opioid; K_i = 11000 nM, δ-opioid)

Table 3. (Continued)

Serotonin receptors

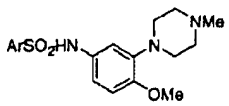
Library: 3.6
Name: Aryloxy-2-propanolamine
Size: Not defined (large)
Affiliation: Merck [229]
Note: Solution-phase parallel synthesis.

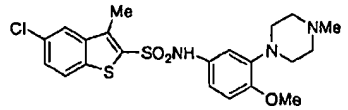




Receptor: 5-HT_{1A} (human receptors in HeLa cells)
Activity: K_i = 1.0 nM

Library: 3.7
Name: Bisaryl sulfonamide
Size: >12 members
Affiliation: SmithKline Beecham [30]
Note: Solution-phase optimization library.

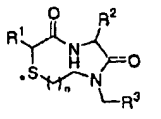


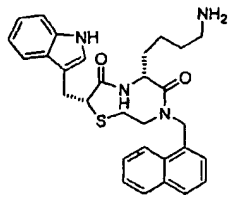


Receptor: 5-HT_6 (human cloned receptors in HeLa cells)
Activity: pK_i = 9.2 (selective vs.13 subtypes and other receptors); pK_b 8.5 (5-HT simulated adenylyl cyclase; antagonist)

Somatostatin receptors

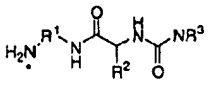
Library: 3.8
Name: β-Turn mimetic
Size: 172 members
Affiliation: Ellman, J. A.; *et al.* [213]

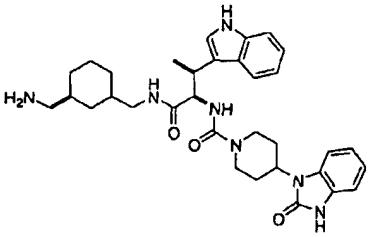




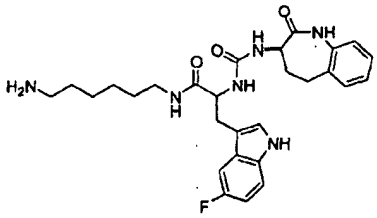
Receptor: Somatostatin-5 ($hsstr_5$, human)
Activity: IC_{50} = 87 nM (ca. 5-fold selective vs. $hsstr_1$; >10-fold selective vs. $hsstr_{2-4}$)

Library: 3.9
Name: Amino acid amides
Size: 131,670
Affiliation: Merck [158]

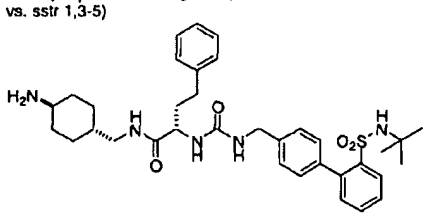




Receptor: Somatostatin-2 (sstr 2; human)
Activity: K_i = 0.04 nM, agonist (>1000x selective vs. sstr 1,3-5)



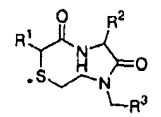
Receptor: Somatostatin-5 (sstr 5; human)
Activity: K_i = 170 nM, agonist (>23x selective vs. sstr 2; >1000x selective vs. sstr 1,3,4)

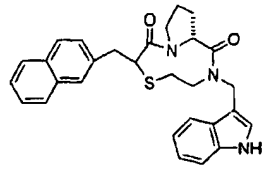


Receptor: Somatostatin-1 (sstr I; human)
Activity: K_i = 64 nM agonist (>23x selective vs. sstr 2-5)

Other receptors

Library: 3.10
Name: β-Turn mimetic
Size: 951 members
Affiliation: Ellman, J. A.; *et al.* [96]
Note: 951 compounds selected from a library of 5544 compounds.

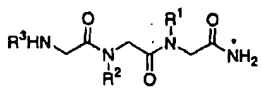


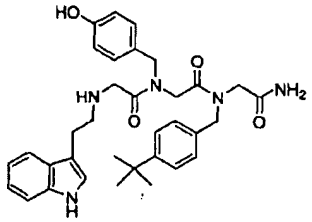


Target: Melanocortin-1
Activity: EC_{50} = 42.5 μM (agonist)

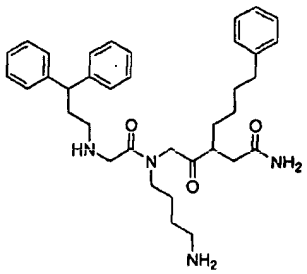
Table 3. (Continued)

Library: 3.11
Name: Tripeptoid
Size: 328,509 members
Affiliation: Eberle, A. N.; *et al.* [99]



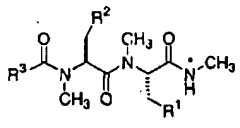


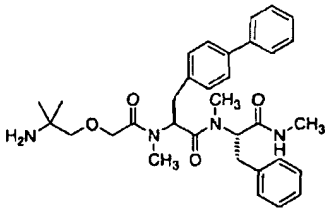
Receptor: GRP/bombesin
Activity: K_D = 3.40 μM



Receptor: Melanocortin-1 (human)
Activity: K_D = 1.58 μM

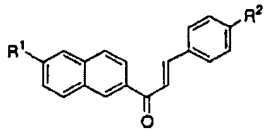
Library: 3.12
Name: Dipeptide
Size: 96 members
Affiliation: Novo Nordisk [5]
Note: Optimization library based on ipamiorelin.

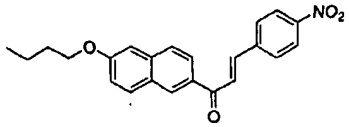




Receptor: Growth hormone segretagogue (rat pituitary cell assay)
Activity: EC_{50} = 1 nM (agonist)

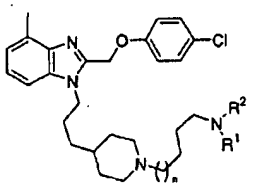
Library: 3.13
Name: Chalcone
Size: ca. 40 members
Affiliation: Natu, A. A.; *et al.* [63]
Note: Solution-phase synthesis of two sets of nine combinatorial mixtures.

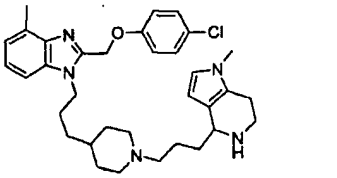




Receptor: Leukotriene B_4 (human whole blood)
Activity: IC_{50} = 18.5 μM (Inhibition of LTB_4 formation)

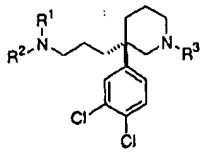
Library: 3.14
Name: Benzimidazole diamine
Size: 84 members
Affiliation: Lilly [205]
Note: Optimization library based on LY344090. Most active compound derived from unexpected Pictet-Spengler cyclization.

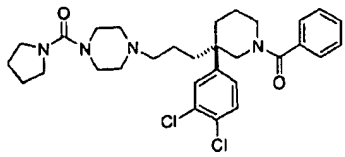




Receptor: Neuropeptide Y-1 (NPY-1; human)
Activity: K_i = 13 nM (antagonist)

Library: 3.15
Name: Dichlorophenyl-3-propylpiperidine
Size: 49 members
Affiliation: SmithKline Beecham [188]
Note: Solution-phase synthesis. Focused library based on Sanofi lead structure.





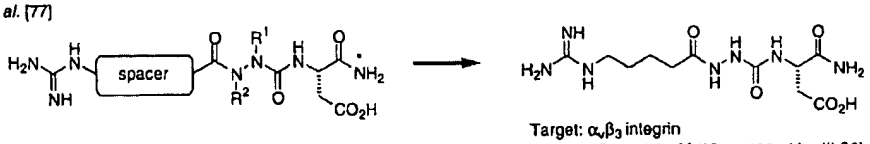
Receptor: Neurokinin-3 (human)
Activity: K_i = 35 nM (antagonist)

[a] Asterisk (*) indicates point of attachment to the resin.

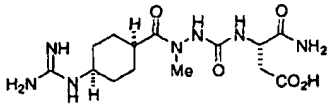
Table 4. Chemical Libraries Targeted for Non-G-Protein Coupled Receptors (non-GPCRs)[a]

Integrins

Library: 4.1
Name: Azapeptoid
Size: 6 members
Affiliation: Kessler, H.; *et al.* [77]

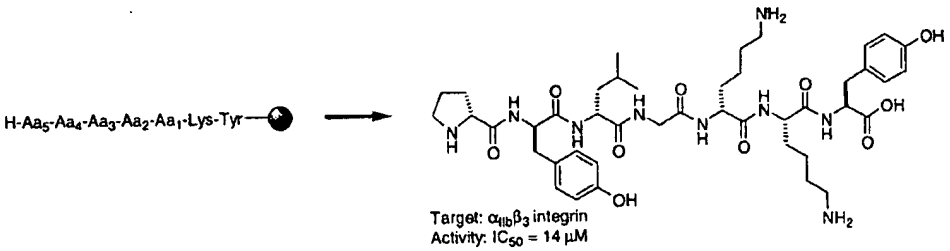


Target: $\alpha_v\beta_3$ integrin
Activity: IC_{50} = 6.8 μM (IC_{50} >100 μM, αIIbβ3)



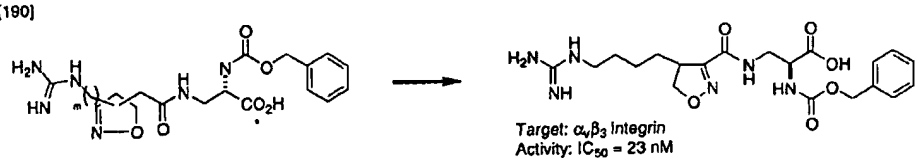
Target: $\alpha_{IIb}\beta_3$
Activity: IC_{50} = 2.7 μM (IC_{50} >100 μM, αvβ3)

Library: 4.2
Name: Pentapetide
Size: Large
Affiliation: Selectide Corp. [223]
Note: L- and D-amino acids was in split-pool synthesis. On-bead screening.



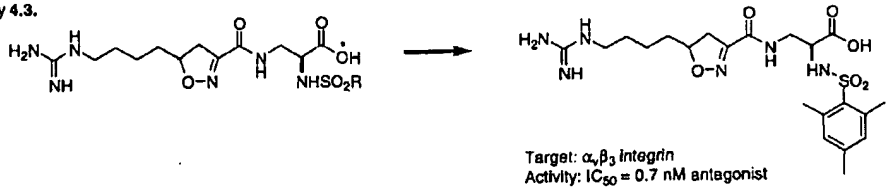
Target: $\alpha_{IIb}\beta_3$ integrin
Activity: IC_{50} = 14 μM

Library: 4.3
Name: Isoxazolinyl guanidine
Size: Not defined
Affiliation: DuPont Pharm. [190]



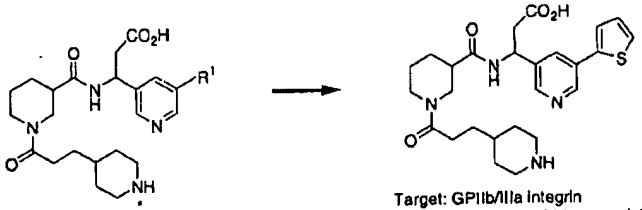
Target: $\alpha_v\beta_3$ Integrin
Activity: IC_{50} = 23 nM

Library: 4.4
Name: Isoxazolinyl guanidine
Size: Not defined
Affiliation: DuPont Pharm. [190]
Note: Optimization library based on lead identified in library 4.3.



Target: $\alpha_v\beta_3$ Integrin
Activity: IC_{50} = 0.7 nM antagonist

Library: 4.5
Name: 5-Substituted pyridine
Size: 15 members
Affiliation: R. W. Johnson Pharm. [104]
Note: Optimization library.

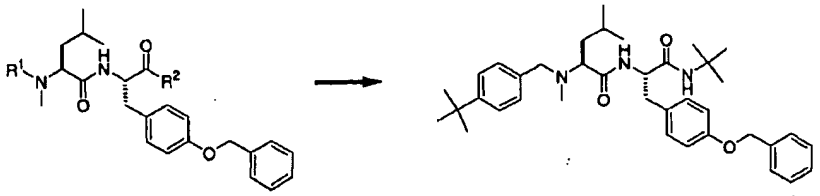


Target: GPIIb/IIIa Integrin
Activity: IC_{50} = 1.5 nM (antagonist)

Table 4. (Continued)

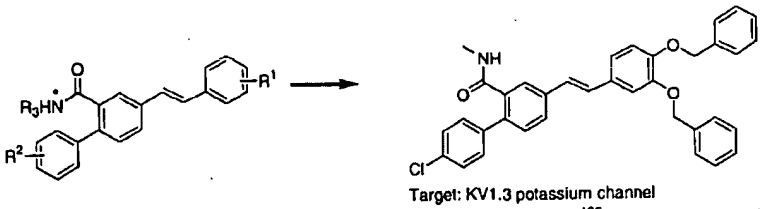
Ion channels and transporters

Library: 4.6
Name: *N,N*-Dialkyldipeptidylamine
Size: 30 members
Affiliation: Parke-Davis [195]
Note: Solution-phase synthesis.



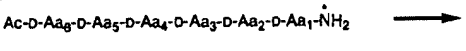
Target: N-Type voltage sensitive calcium channel (in IMR-32 cells)
Activity: IC_{50} = 40 nM

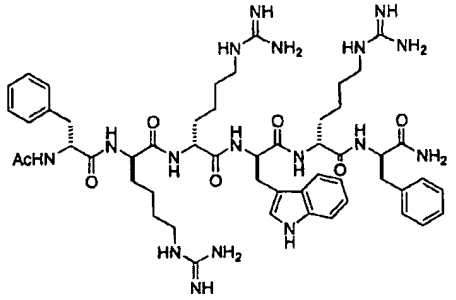
Library: 4.7
Name: Substituted phenyl stilbene
Size: ca. 400 members
Affiliation: Chamberlin, A. R.; *et al.* [145]
Note: Kenner's safety-catch linker used.



Target: KV1.3 potassium channel
Activity: IC_{50} = 2.9 μM (^{125}I-ChTx binding assay)

Library: 4.8
Name: D-Hexapeptide
Size: ca. 400 members
Affiliation: Rothman, R. B.; *et al.* [192]
Note: Kenner's safety-catch linker used.

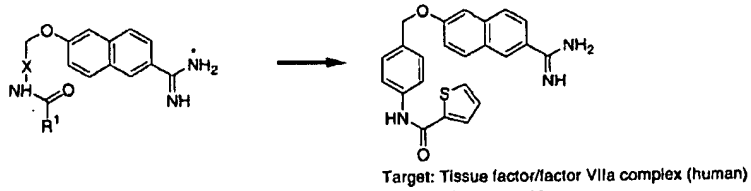




Target: Dopamine transporter
Activity: IC_{50} = 1.8 μM

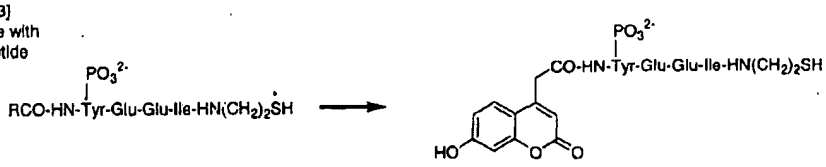
Other non-GPCR

Library: 4.9
Name: Amidinonaphthyl ether
Size: ca. 145 members
Affiliation: Bradley, M.; *et al.* [194]



Target: Tissue factor/factor VIIa complex (human)
Activity: IC_{50} = 4.1 μM

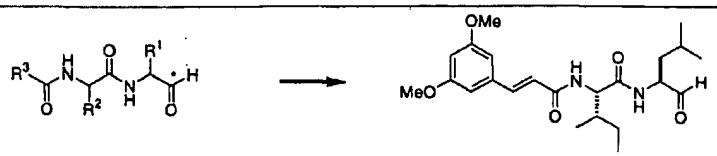
Library: 4.10
Name: Phosphopeptide
Size: 900 members
Affiliation: Lawrence, D. S.; *et al.* [143]
Note: Disulfide link to resin. Cleavage with dithiothreitol in Tris buffer to give peptide conjugates in assay-ready solution.



Target: Src SH2 domain of Lck
Activity: K_D = 35 nM (K_D = 150 nM, Fyn)

Table 4. (Continued)

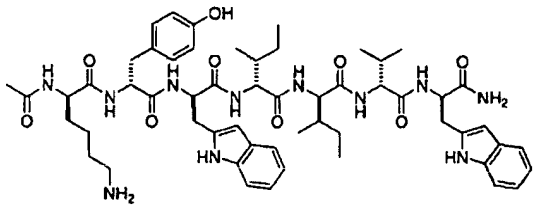
Library: 4.11
Name: Dipeptide aldehyde
Size: 100 members
Affiliation: Scios Inc. [102]



Target: β-Amyloid production
Activity: IC_{50} = 9.6 μM

Library: 4.12
Name: D-Hexapeptides
Size: 47,000,000 members
Affiliation: Centocor, Inc. [127]
Note: Positional scanning protocol using D-amino acids exclusively.

Ac-D-Aa_6-D-Aa_5-D-Aa_4-D-Aa_3-D-Aa_2-D-Aa_1-NH_2



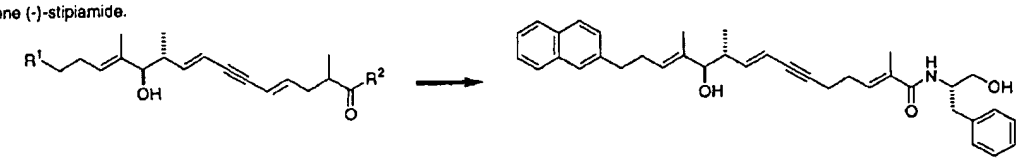
Target: TNFα
Activity: IC_{50} = 0.33 μM (antagonist effect due to binding of hexapeptide to TNF-α not its receptor)

[a] Asterisk (*) indicates point of attachment to the resin.

Table 5. Chemical Libraries Displaying Cytotoxic and Antiinfective Activity[a]

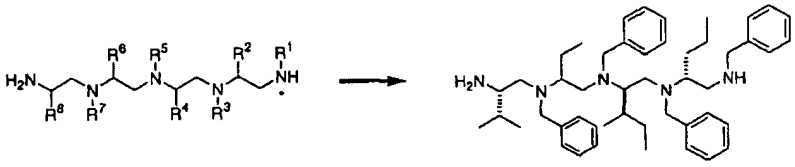
Cytotoxic agents

Library: 5.1
Name: Polyene
Size: 42 members
Affiliation: Amdris, M. B.; *et al.* [4]
Note: Solution-phase indexed combinatorial library based on MDR reversing polyene (-)-stipiamide.



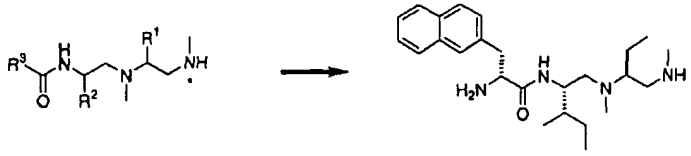
Target: McF7 - adriamycin resistant cells expressing P-glycoprotein (multidrug resistant pump)
Activity: IC_{50} = 1.45 μM

Library: 5.2
Name: Pentamine
Size: ca. 7,311,616 members
Affiliation: Appel, J. R.; *et al.* [7]



Target: 60 different cell lines
Activity: $MGIC_{50}$ = 2.02 μM (mean growth inhibition)

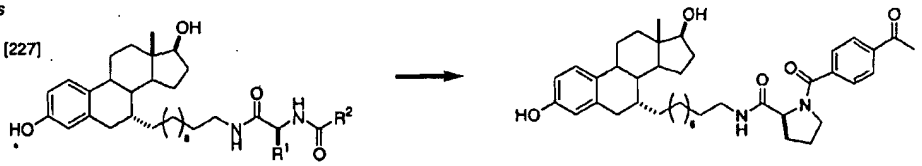
Library: 5.3
Name: *N*-Acylated triamine
Size: 454,272 members
Affiliation: Appel, J. R.; *et al.* [7]



Target: 60 different cell lines
Activity: $MGIC_{50}$ = 0.69 μM
(mean growth inhibition)

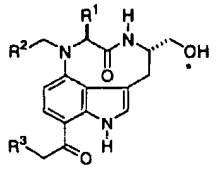
Table 5. (Continued)

Library: 5.4
Name: *Estradiol derivatives*
Size: 20 members
Affiliation: Poirier, D.; *et al.* [227]



Target: T-47D (human breast cancer cell)
Activity: 95% antiproliferative activity at 0.1 μM

Library: 5.5
Name: Indole lactams
Size: 31 members
Affiliation: Waldmann, H.; *et al.* [156]
Note: Library based on known PKC activator. (-)-indolactam V.

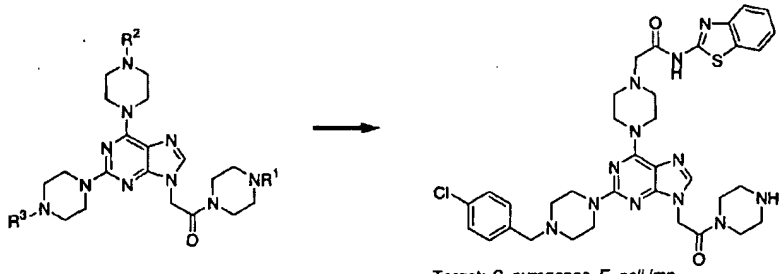


No single compound identified

Target: PKC activation in Swiss 3T3 cells
Activity: 3-5 fold increase in MARKS translocation at 200 nM; less efficient than (-)-indolactam V.

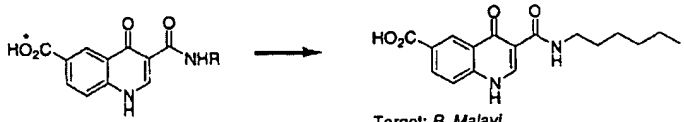
Antiinfective agents

Library: 5.6
Name: Substituted purine
Size: 2725 members
Affiliation: Isis Pharm. [71]
Note: Solution-phase simultaneous addition of functionalities.



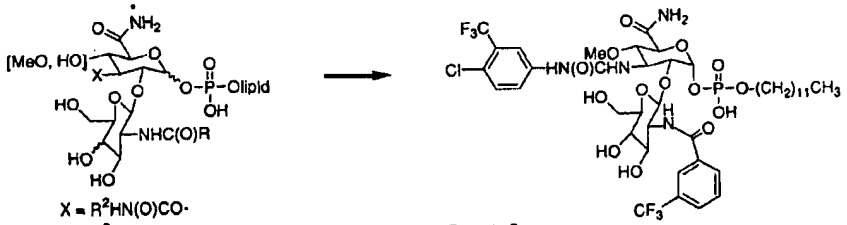
Target: *S. pyrogenes, E. coli Imp-*
Activity: "potent broad-based antibacterial profile"

Library: 5.7
Name: Quinolone
Size: 5 members
Affiliation: Chauhan, P. M. S.; *et al.* [215]



Target: *B. Malayi*
Activity: EC_{100} = 100 μM

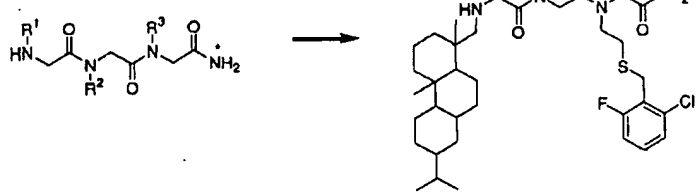
Library: 5.8
Name: Disaccharide
Size: 1300 members
Affiliation: Intercardia Inc. [212]
Note: IRORI R_f tags.



X = $R^2HN(O)CO$-
X = $R^2HN(O)CHN$-

Target: *S. aureus*
Activity: MIC = 6.25 μg/mL

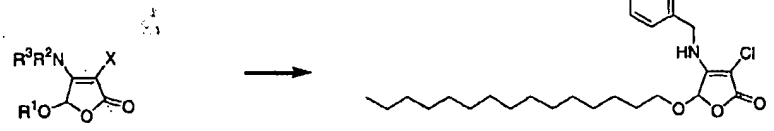
Library: 5.9
Name: Peptoid
Size: 845 members
Affiliation: Chiron Corp. [81,165]
Note: Optimization library.



Target: *E. faecium*
Activity: MIC = 5 μg/mL; broad spectrum

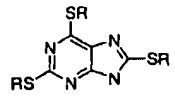
Table 5. (Continued)

Library: 5.10
Name: Butenolide
Size: 288 members
Affiliation: Lattmann, E.; *et al.* [134, 135]
Note: Solution-phase synthesis from commercially available halogenated mucochloric acids.



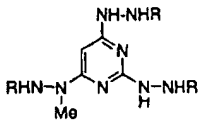
Target: *S. aureus (MRSA 96-7778)*
Activity: MIC = 8-16 μg/mL

Library: 5.11
Name: Substituted purine
Size: Not defined
Affiliation: Isis Pharm. [119]
Note: Solution-phase simultaneous addition of functionalities.



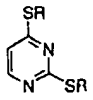
Target: Bacteria
Activity: Not defined

Library: 5.12
Name: Substituted pyrimidine
Size: Not defined
Affiliation: Isis Pharm. [119]
Note: Solution-phase simultaneous addition of functionality.



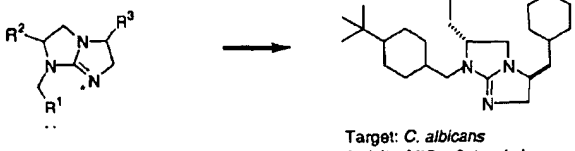
Target: Bacteria
Activity: Not defined

Library: 5.13
Name: Substituted pyrimidine
Size: Not defined
Affiliation: Isis Pharm. [119]
Note: Solution-phase simultaneous addition of functionality.



Target: Bacteria
Activity: Not defined

Library: 5.14
Name: Bicyclic guanidine
Size: ca. 100,000 members
Affiliation: Blondelle, S.E.; *et al.* [19]
Note: Positional scanning protocol.



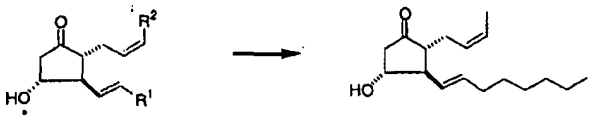
Target: *C. albicans*
Activity: MIC = 3-4 μg/mL

Library: 5.15
Name: Kramerixin analog
Size: ca. 120 members
Affiliation: Fecik, R. A. *et al.* [68]



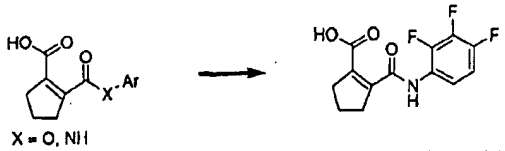
Target: *C. albicans*
Activity: MIC = 1.25 μg/mL

Library: 5.16
Name: Prostanoid
Size: ca. 64 members
Affiliation: Janda, K. D.; *et al.* [141]
Note: Library created on soluble support.



Target: Cytomegalovirus (CMV)
Activity: 98% reduction in viral titer (control titer: 6.8×10^3 PFU/mL (plaque forming units))

Library: 5.17
Name: Cyclopentene-1,2-dicarboxylic acid derivative
Size: 600 virtual members
Affiliation: de Julian-Ortiz, J. V.; *et al.* [60]
Note: Compounds identified through process of virtual library synthesis and computational screening.

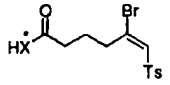


Target: HSV-1 (plaque inhibition assay)
Activity: IC_{50} = 0.9 μM

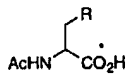
[a] Asterisk (*) indicates point of attachment to the resin.

Table 6. Scaffold Derivatization: (a) Solid Phase, (b) Solution Phase[a]

(a) Solid phase



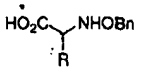
- Glaxo Wellcome [36]
- 5 ex; 24-95%
- addition of TsBr to resin-bound alkyne and alkenes; X = NR, O



- Ylm, A.-M. [242]
- 3 ex; 49-60%
- radical addition of organomercury chlorides to dehydroalanine



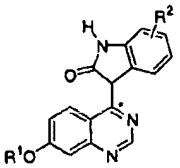
- RPR [197]
- 22 ex; 0-54%
- reductive cleavage of resin-bound Weinreb type amides



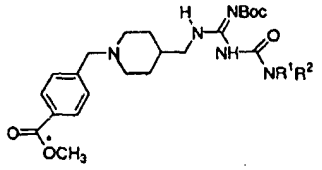
- Kobe Pharm. [158]
- 7 ex; 24-83%
- triethylborane-mediated radical addition of alkyls to glyoxylic oxime ethers



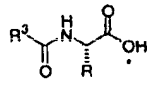
- Waldermann, H. [217]
- 7 ex; 50-95%
- traceless linker based on acyl hydrazines



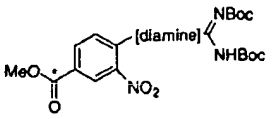
- Zeneca [100]
- 13 ex; 0-72%
- nucleophilic displacement of resin-bound quinazolines with oxindols



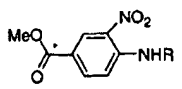
- Sun, C.-M. [218]
- 10 ex; 75-92%
- aminolysis of bis-Boc guanidines then acylation; liquid phase synthesis



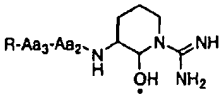
- Petillo, P. A. [51]
- 7 ex; high purity
- $MeSiCl_3$-mediated conversion of Fmoc-amino acid to isocyanate, then addition of amines



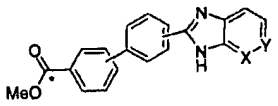
- Sun, C.-M. [103]
- 3 ex; ca. 84%
- from soluble polymer-bound 4-fluoro-3-nitro benzoic acid



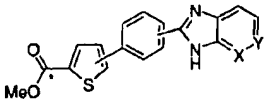
- Sun, C.-M. [175]
- 8 ex; 99%
- amine displacement of soluble-polymer supported 4-fluoro-3-nitrobenzoic acid



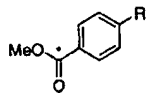
- Corvas [206]
- 20 ex; 17-83%
- from resin-bound argininal equivalent



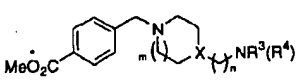
- Schotten, T. [17]
- 19 ex; 33-60%
- liquid-phase Suzuki coupling



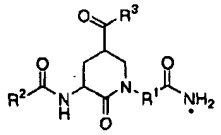
- Schotten, T. [17]
- 6 ex; 54-93%
- liquid-phase Suzuki coupling



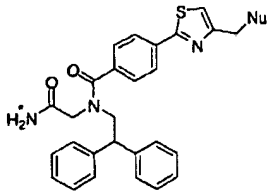
- Kondo, Y. [126]
- ca. 5 ex;
- immobilized organometallic reagents; R = electrophile, Ph



- Sun, C.-M. [203]
- 15 ex; 80-95%
- from resin-bound 4-chloro-methylbenzoic acid; liquid-phase synthesis; X = CH, N; n = 0, 1; m = 1, 2



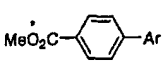
- Glaxo Wellcome [147]
- size not defined
- ^{1}NH alkylation of 3-amino-5-carbomethoxy-1H-pyridin-2-one to resin-bound halo acids, then N-acylation and amination



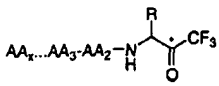
- Woski, A. [41]
- displacement of Cl in resin-bound chloromethylthiazole



- Brase, S. [28]
- ca. 9 ex; good yield
- triazene linker



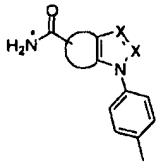
- Flinn, M. G. [29]
- 7 ex; 0-95%
- Stille coupling with resin-bound aryl stannanes



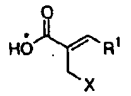
- Boehringer Ingel. [183]
- 100 members
- derivatization of Boc-protected trifluoromethyl ketones attached to resin via semicarbazone linkage



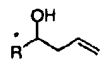
- Morishima, H. [220]
- 9 ex; 90->95%
- Curtius rearrangement and trapping intermediate isocyanate with Wang resin, then hydrolysis



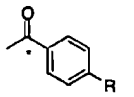
- Du Pont [54]
- 4 ex; 55-64%
- C-N cross-coupling X = CH, N



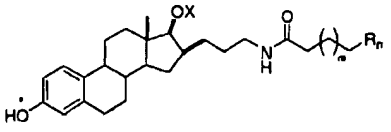
- Jung, G. [189]
- 5 ex; >65%
- from Baylis-Hillman intermediate; X = OAr, NR^2R^3



- Pharmacopeia [39]
- 10 ex; ca. 70%
- addition of allylindium or allylboronate to resin-bound aldehyde



- Schering AG [111]
- 7 ex; 28-59%
- Suzuki coupling of resin-bound 4-bromobenzophenone dithioketal

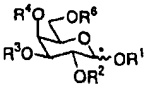


- Poirier, D. [226]
- 2 ex; 20-30%
- from resin-bound 16-β-(azidopropyl)estradiol; X = H, COR

ROH

- Hanessian, S. [94]
- 3 ex; 94-98%
- from 2-pyridylthiocarbonate resin, ROH, AgOTf, then TFA.

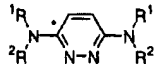
Table 6. (Continued)



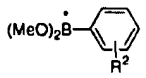
- Kunz, H. [115]
- 14 ex; 6-69%
- from resin-bound orthogonal protected galactose



- Abell, C. [224]
- 10 ex; 58-96%
- esters and amides from novel safety-catch linker involving formation of N-arylindoles on resin; X = OR, NRR



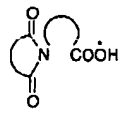
- Hilbert, M. [177]
- 8 ex; 50-100%
- functionalization of 3,6-dichloropyridazines



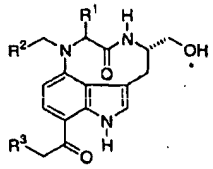
- Carboni, B. [38]
- 6 ex; 54-67%
- functionalization of resin-bound boronic acids, then transesterification



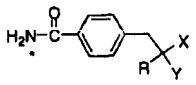
- Carboni, B. [38]
- 3 ex; 63-74%
- oxidative cleavage of resin-bound aryl boronic acids



- Chandrasekhar, S. [42]
- 6 ex; 58-72%
- reaction of resin-bound amino acids with anhydrides



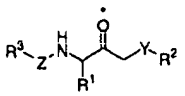
- Waldmann, H. [156]
- 31 ex; 10-65 ex
- derivatives of (-)-Indolactam V; THP linker



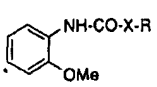
- PE Biosystems [45]
- 5 members
- C-C bond formation via Mitsunobu reaction of resin-bound alcohols and active methylene compounds



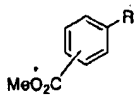
- Taddei, M. [152]
- 15 ex; 58-87%
- activation of carboxylic acid via resin-bound 2,4-dichlorotriazine



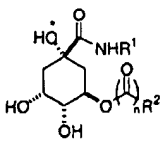
- Ellman, J. A. [138]
- 8 ex; 40-100%
- acylhydrazone linkage strategy; Z = CO, SO_2, Aa; Y = O, S, NH, NR



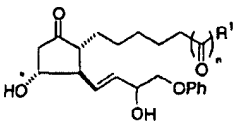
- *Langlois, M.* [58]
- 11 ex; 53-90%
- use of traceless silicone linker; BOC deprotection with β-catecholborane



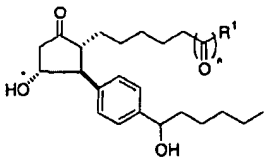
- Janda, K. D. [204]
- 15 ex; 69-99%
- Stille coupling on soluble polymer



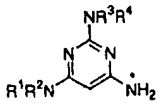
- *Abell, C., W.* [182]
- 12 ex; 62-92%
- derived from D-(-)-quinic acid; n = 0, 1



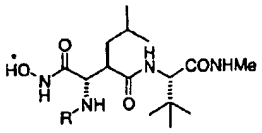
- Ellman, J. A. [65]
- 13 ex; 18-47%
- Side chains introduced via Suzuki and Michael reactions



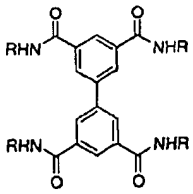
- Ellman, J. A. [65]
- 13 ex; 18-47%
- Side chains introduced via Suzuki and Michael reactions



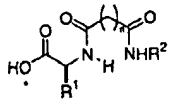
- Bradley, M. [85]
- ca. 17 ex; 11-52%
- attachment of 4,6-dichloro-2-thiomethylpyrimidine to Rink resin and sequential amination



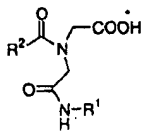
- Zeneca [10]
- ca. 30 ex; 43-100%
- attachment of dipeptide acid (R = Fmoc) to sasrin-ONH_2, deprotection and N-derivatization; R = RCO, RCO_2, RCH_2



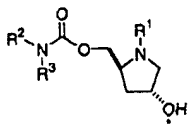
- Boger, D. L. [20]
- *ca.* 15 ex; 75-95%
- Coupling using 10% Pd/C, Et_3N, DMF, 100 °C, 16 h



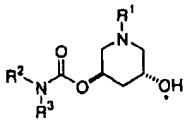
- Biochem. Pharm. [234]
- 576 members
- Pre-activation of symmetric dicarboxylic acids with BOP, then additon to resin-bound amino acid



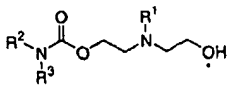
- CombiChem [48]
- 46 ex; 13-73%
- derived from iminodiacetic acid; use of soluble polymer



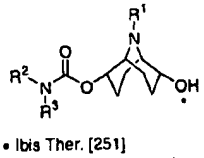
- Ibis Ther. [251]
- part of a 17,000 member library



- Ibis Ther. [251]
- part of a 17,000 member library



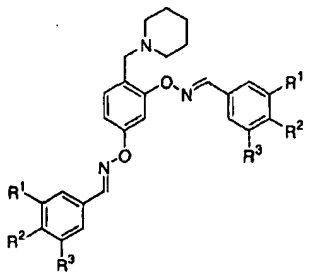
- Ibis Ther. [251]
- part of a 17,000 member library



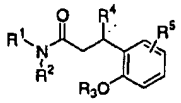
- Ibis Ther. [251]
- part of a 17,000 member library

Table 6. (Continued)

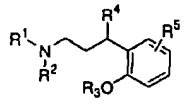
(b) Solution phase



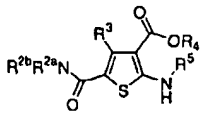
- Eliseev, A. V. [161]
- 220 members
- pair-wise condensation of ArCHO with 2,4-diaminoxyaryl



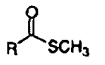
- R. W. Johnson [35]
- 300 members
- aminolysis of dihydrocoumarins



- R. W. Johnson [35]
- 5 ex; 51-84%
- aminolysis of dihydrocoumarin, then borane reduction



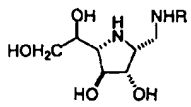
- Cadus [154]
- 3000 member library
- thiophene template derived from three-component condensation; R^3 = Me or CH_2OMe



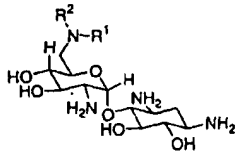
- Vulfson, E. N. [120]
- 11 ex; high purity
- from carboxylic acids, methyl chlorothioformate and catalytic DMAP

Ar-OTf (ONf)

- Zhu, J. [23]
- 20 ex; 65-100%
- Perfluoroalkanesulfonyl transfer with polymer supported base



- Fleet, G. W. J. [142]
- 3 ex; ca. 35-50%
- derived from amino lactone

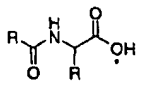


- Berrisford, D. J. [167]
- 15 ex; 26-60%
- from neamine

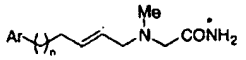
[a] Asterisk (*) indicates point of attachment to the resin.

Table 7. Acyclic Synthesis: (a) Solid Phase, (b) Solution Phase[a]

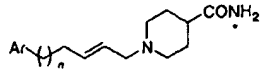
(a) Solid phase



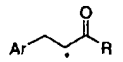
- O'Donnell, M. J. [168,169]
- 14 ex; 51-99%
- reaction of resin-bound Schiff base with organo boranes



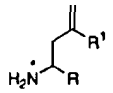
- Hoffmann-La Roche [236]
- 2 ex; 84-87%
- Pd-catalyzed three-component coupling



- Hoffmann-La Roche [236]
- 13 ex; 70-95%
- Pd-catalyzed three-component coupling



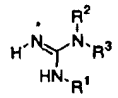
- Ganesan, A. [131]
- 26 ex; 0-49%
- sequential Baylis-Hillman and Heck reactions of resin-bound acrylic acids, then decarboxylation



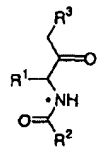
- Hiemstra, H. [155]
- ca. 40 members
- three-component condensation of resin-bound carbamate, RCHO and allylsilanes



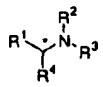
- Kobayashi, S. [6]
- 16 ex; 41-93%
- reductive alkylation of BOBA resin then acylation



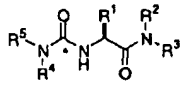
- P&G Pharm. [238]
- 8 ex; 38-74%
- use of a novel traceless linker: acyl isothiocyanate resin



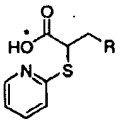
- Nielsen, J. [105]
- 18 members
- microwave assisted Ugi four-component condensation



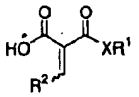
- Katritzky, A. R. [117]
- 5 ex; good yields
- from polymer-bound 1*H*-benzotriazole



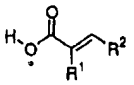
- Du Pont [92]
- 10 ex; 45-100%
- phoxime resin



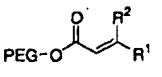
- Ganesan, A. [249]
- 5 ex; 32-94%
- intermolecular alkyl radical conjugate addition of resin-bound acrylate



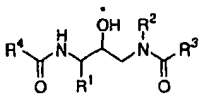
- Monsanto [91]
- 96 members
- from resin-bound malonic acid



- RPR [196]
- 48 ex; 62->95%
- Horner-Emmons olefination with resin-bound phosphonate esters

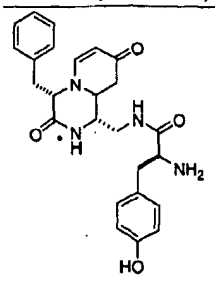


- Blettner, C. G. [18]
- 14 ex; 88-98%
- liquid-phase Horner-Emmons olefination with polymer-bound ketophosphonate and RCHO, then Heck reaction with R^2-I

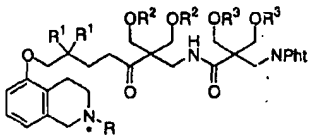


- *CombiChem* [248]
- 92 members
- THP linker

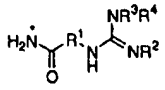
Table 7. (Continued)



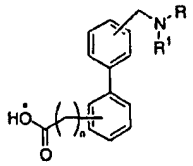
• Goodman, M. [57]
• 1 ex; 45%
[4+2] cyclocondensation of resin-bound imine with Danishefsky diene, then intracyclative cleavage



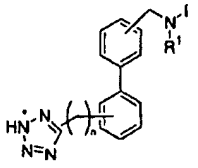
• Heinonen, P. [98]
• 96 member library
• coupling of α,α-disubstituted β-alanine units to resin-bound tetrahydroisoquinoline



• Merck [43]
• 48 ex; 11-77%
• synthesis carried out on multivalent soluble support

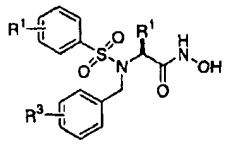


• Tripos [56]
• >150 members
• R_f tags

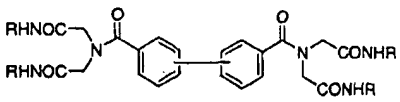


• Tripos [56]
• >100 members
• R_f tags

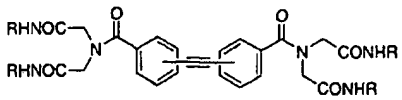
(b) Solution phase



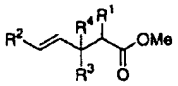
• Ley, S. V. [37]
• 27 ex; ca. 50%
• polymer-supported reagents and scavenger resins



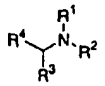
• Boger, D. L. [21]
• 64,980 members
• from N-Boc iminodiacetic acid anhydride



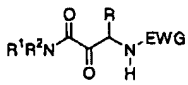
• Boger, D. L. [21]
• 64,980 members
• from N-Boc iminodiacetic acid anhydride



• Argonaut [107]
• 5 ex; 0-60%
• ester enolate Claisen rearrangement using polymer-supported silyl triflate



• Schowalter, H. D. H. [199]
• 12 ex; 20-77%
• amines from Mannich adducts of polymer-supported benzotriazole



• Chiron [121]
• 10 ex; 61-85%
• ring opening of 3,3-di-OMe-N-sulfonyl and carbamoyl azetidin-2-ones, then ketal hydrolysis

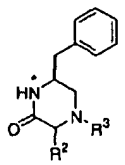


• Glaxo Wellcome [173]
• ca. 21 ex; 20-50%
• resin-bound from benzotriazoles, aldehydes, and amines and alcohols; X = OR^3; NR^3R^4

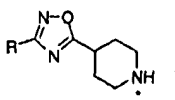
[a] Asterisk (*) indicates point of attachment to the resin.

Table 8. Monocyclic Synthesis: (a) Solid Phase, (b) Solution Phase[a]

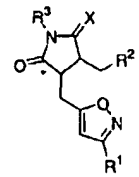
(a) Solid phase



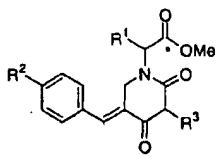
• Ibis [132]
• 4 ex; 61-90%
• ring formation via intramolecular Mitsunobu reaction of resin-bound phenylalaninol



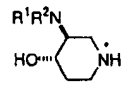
• Merck [146]
• 7 ex; 75-99%
• base-catalyzed condensation-cyclization of *resin-bound esters and* amidoximes



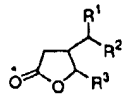
• Kurth, M. J. [176]
• 18 ex; 20-39%
• dipolar cycloaddition on resin; intracyclative cleavage; X = O, S



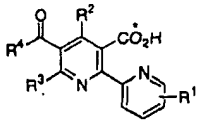
• R. W. Johnson [40]
• 5 ex; 60-72%
• Dieckmann condensation



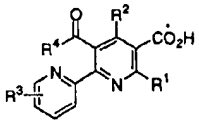
• Hoffman-La Roche [191]
• 4 ex; 53-78%
• aminolysis of resin-bound epoxide; plus regioisomer



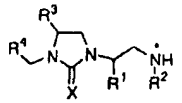
• Toru, T. [237]
• 12 ex; 47-93%
• Bu_3SnH-mediated intramolecular cyclization of resin-bound β-bromoethylacetals, then Jones oxidation



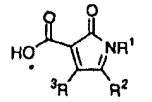
• Affymax [222]
• 7 ex; ca. 30%
• Knoevenagel-Hantzsch reaction sequence



• Affymax [222]
• 4 ex; ca. 30%
• Knoevenagel-Hantzsch reaction sequence

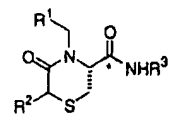


• Houghten, R. A. [164]
• 4 libraries of 118,400 members each
• $CXIm_2$-mediated ring formation from resin-bound polyamine; X = O, S

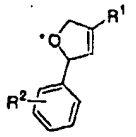


• Monsanto [157]
• 11 ex; 43-80%
• from resin-bound malonic acid and amino acid alcohols

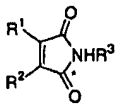
Table 8. (Continued)



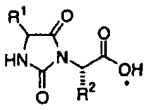
- Affymax [160]
- 7 ex; 17-65%
- intramolecular cyclization of cysteine SH and 2-bromoamide carboxylic acids, then post cleavage amidation



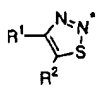
- Knochel, P. [193]
- 20 ex; 55-95%
- formation of resin-bound Grignard, addition of ArCHO, then intracyclative cleavage



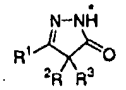
- Organon [12]
- 2 ex; 24-57%
- intracyclative cleavage of resin-bound 1,2-dicarboxylic acids



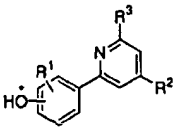
- Petillo, P. A. [58]
- 7 ex; good purity
- direct cyclization of Fmoc-dipeptides via treatment with Et_3N/TMSCl and heating



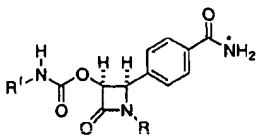
- Argonaut [106]
- 6 ex; 48-98%
- $SOCl_2$-mediated cyclative cleavage of resin-bound sulfonyl-hydrazones



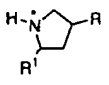
- Kobayashi, S. [125]
- 18 ex; 38-88%
- $Sc(OTf)_3$-mediated Mannich type reaction of resin-bound acylhydrazones and ketene silyl acetals, then NaOMe-mediated cyclative cleavage



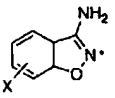
- Wyeth-Ayerst [50]
- 10 ex; 19-62%
- from resin-bound hydroxyacetophenones



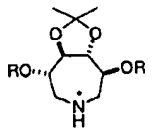
- Chiron [207]
- 12 ex; 78-89%
- Staudinger reaction using resin-bound arylimines



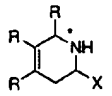
- Hiemstra, H. [231]
- 6 ex; 36-98%
- N-acyliminium ion cyclization



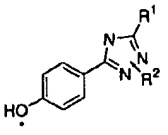
- Eli Lilly [144]
- 12 ex; 55-86%
- reaction of 2-fluoro-benzonitriles with oxime resin cleavage followed by intracyclative cleavage



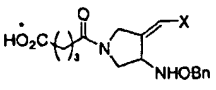
- Merrer, Y. L. [76]
- 7 ex; 53-90%
- alkylation of Rink resin with L-iditol bis-epoxide then OH-derivatization; R = H, COR, CONHR, $COCH_2NHFmoc$



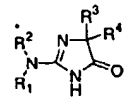
- Wang, G. [246]
- 8 ex; 52-96%
- Yb (OTf), -catalyzed aza Diels-Alder reaction with resin-bound imines; cleavage using ACE chloride; X = H, CO_2Et,



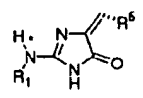
- Katritzky, A. R. [118]
- 13 ex; >95%
- reaction of resin-bound acylhydrazine with amidines, then optionally N-alkylation



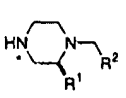
- Kobe Pharm. [159]
- 3 ex; 64-77%
- Et_3B-mediated intramolecular radical cyclization of oxime ethers



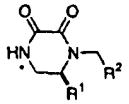
- Tularik [72]
- 8 ex; 86-97%
- reaction of resin-bound S-methyl isothioureas with Fmoc-amino acids



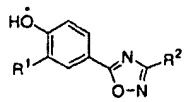
- Tularik [72]
- 5 ex; 46-91%
- reaction of resin-bound S-methyl isothioureas with oxazolones



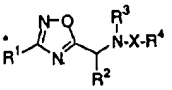
- Houghten, R. A. [162]
- 12 ex; >80%
- reduction of 1,2-diketo-piperazines derived from reduced N-acylated amino acids



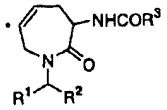
- Houghten, R. A. [162]
- 12 ex; >80%
- from resin-bound reduced N-acylated amino acids



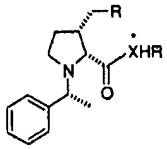
- Novo Nordisk [198]
- 16 ex; 24-40%
- cyclization of resin-bound acylated N-hydroxyamidines



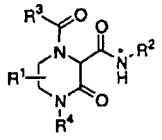
- *Trega* [97]
- size & yields not given
- conversion of resin-bound nitriles to amide oximes and cyclization to oxadiazoles using N-protected amino acid anhydrides; X = CO, SO_2



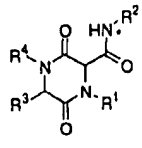
- Amgen [183]
- ca. 15 ex; 15-60%
- ring closing metathesis



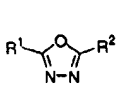
- Perrotta, E. [116]
- ca. 5 ex; high yield
- amino-zinc-enolate cyclization then derivatization



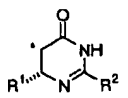
- RPR [109]
- 6 ex; 31-100%
- Ugi three-component condensation with ethyl-glyoxalate



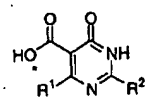
- RPR [109]
- 3 ex; 70-100%
- Ugi three-component condensation with ethyl-glyoxalate



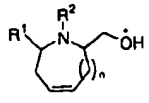
- Novartis [26]
- 16 ex; 75-98%
- dehydration of 1,2-diacylhydrazines using polymer-supported Burgess reagent



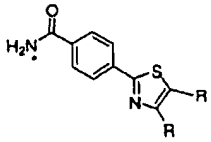
- Monsanto [90]
- 7 ex; 0-21%
- Knoevenagel condensation of resin-bound malonic ester, condensation with amidines then decarboxylation



- Monsanto [90]
- 10 ex; 49-99%
- Knoevenagel condensation of resin-bound malonic ester, condensation with amidines then oxidation with CAN

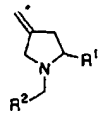


- Blechert, S. [181]
- 4 ex; > 70%
- ring formation via olefin metathesis

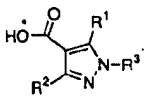


- *Chiron* [78]
- 4 ex; 54-97%
- Hantzch thiazole synthesis; on-resin conversion of ArCN to thioamides

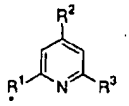
Table 8. (Continued)



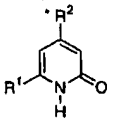
- Brown, R. C. D. [32]
- ca. 7 ex; 16-95%
- Imino-Sakurai and Pd-catalyzed intracyclative cleavage



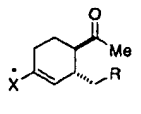
- Jung, G. [83]
- 13 ex; 41-61%
- from alkylidene-and arylidene-β-oxo esters



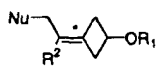
- Jung, G. [83]
- 9 ex; 72-85%
- condensation of resin-bound enones and 1-(methoxycarbonylmethyl) pyridinium bromide



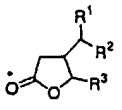
- Jung, G. [83]
- 6 ex; 71-75%
- condensation of resin-bound enones with 1-(methoxycarbonylmethyl) pyridinium bromide



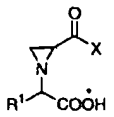
- Blechert, S. [200]
- 5 ex; 26-55%
- yne-ene cross metathesis and Diels-Alder cycloaddition then intracyclative cleavage



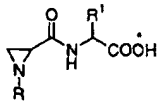
- Kurth, M. J. [49]
- 8 ex; 30-38%
- derived from resin-bound allyl sulfones



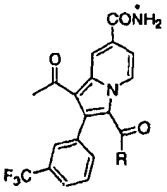
- Toru, T. [237]
- 6 ex; 47-93%
- radical cyclization of resin-bound β-bromoethylacetals



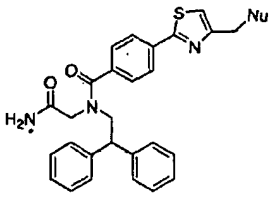
- Taddei, M. [70]
- ca. 10 ex; good yields
- Gabriel-Cromwell synthesis from resin-bound amino acid



- Taddei, M. [70]
- ca. 20 ex; good yields
- Gabriel-Cromwell synthesis from resin-bound α-bromoacrylamide



- Chiron [79]
- 9 ex; ca. 50%
- from resin-bound isonicotinic acid



- Chiron [78]
- 8 ex; 58-95%
- Hantzch thiazole synthesis; on-resin conversion of ArCN to thioamides

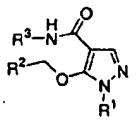
(b) Solution phase



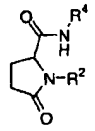
- Ganesan, A. [130]
- 10 ex; 25-50%
- reaction of polymer-supported TosMIC with ArCHO



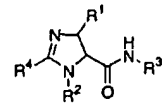
- Ganesan, A. [130]
- 13 ex; 54-85%
- condensation of TosMIC and ArCHO catalyzed by ion exchange resin



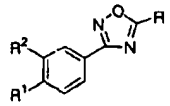
- SKB [31]
- 960 members
- from 5 unique pyrazolinone carboxylic acids



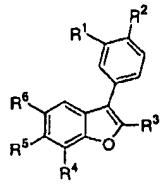
- RepliGen [245]
- 13 ex; 85-100%
- Ugi three-component condensation



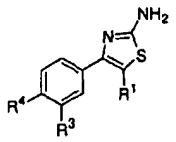
- RPR [110]
- 10,members
- Ugi four-component condensation with Boc-amino acid aldehydes, then TFA-mediated cyclization



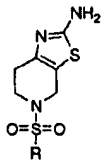
- Argonaut [61]
- 20 ex; 50-69%
- CDI-mediated formation and cyclodehydration of *O*-acylbenzamidoximes



- Ley, S. V. [88]
- 27 ex; 30-95%
- from acetophenones; use of polymer supported reagents



- Ley, S. V. [87]
- 4 ex; 47-95%
- use of scavenger reagent to mediate cyclization of α-bromoketones and thiourea

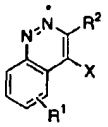


- Ley, S. V. [87]
- 4 ex; 73-95%
- use of scavenger reagent to mediate cyclization of α-bromoketones and thiourea

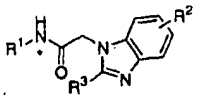
[a] Asterisk (*) indicates point of attachment to the resin.

Table 9. Bicyclic and Spirocyclic Synthesis: (a) Solid Phase, (b) Solution Phase[a]

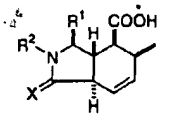
(a) Solid phase



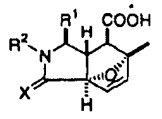
- Brase, S. [27]
- 5 ex; 47-95%
- Pd-catalyzed alkynylation of triazene bound *o*-halo arenes, HX-mediated cleavage and cyclization



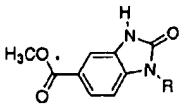
- Affymax [228]
- 12 ex; 43-80%
- nitroarylation of resin-bound glycine, nitro reduction and cyclization with R^3CHO



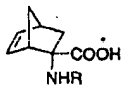
- R. W. Johnson [219]
- 5 ex; 32-55%
- intramolecular Diels-Alder reaction; X = O, 2H



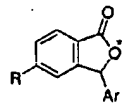
- R. W. Johnson [219]
- 5 ex; 32-55%
- intramolecular Diels-Alder reaction; X = O, 2H



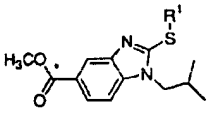
- Sun, C.-M. [174]
- 5 ex; 75-96%
- from resin-bound 4-fluoro-3-nitrobenzoic acid



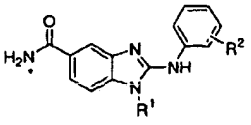
- Burkett, B. A. [34]
- 4 ex; 72-81%
- Diels-Alder reaction between resin-bound dihydroalanine and cyclopentadiene



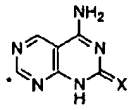
- Novo Nordisk [75]
- 8 ex; 66-81%
- resin-bound benzamide ortho-lithiation then reaction with ArCHO and intracyclative cleavage



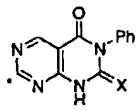
- Sun, C.-M. [240]
- 12 ex; 72-99%
- liquid-phase synthesis from immobilized 4-*fluoro*-3-nitrobenzoic acid



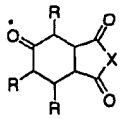
- SIDDCO [210]
- 96 ex; ca. 77%
- from resin-bound 4-*fluoro*-3-*nitro*benzoic acid



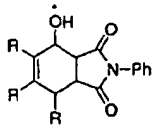
- Chauhan, P. M. S. [214]
- 2 ex; 76-80%
- from resin-bound 2-(alkylthio)-4-aminopyrimidine-5-carbonitrile; resin cleavage with Ni/H_2; X = O, S



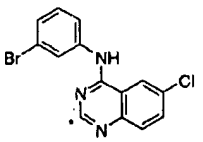
- Chauhan, P. M. S. [214]
- 2 ex; ca. 75%
- from resin-bound 2-(alkylthio)-4-aminopyrimidine-5-carboxamide; resin cleavage with Ni/H_2; X = O, S



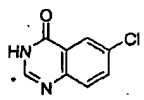
- Du Pont [208]
- 8 ex; 18-99%
- Diels-Alder reaction of resin-bound silyloxydienes derived from polymer-supported silyl triflate and unsaturated ketone; X = O, NPh



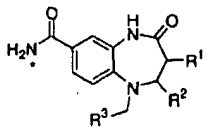
- Du Pont [208]
- 4 ex; 31-97%
- Diels-Alder reactions of resin-bound silyloxydienes derived from polymer-supported silyl triflate and unsaturated aldehyde



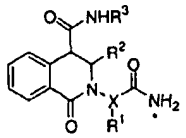
- Abell, C. [53]
- 1 ex; 69%
- reaction of 3-bromoaniline and resin-bound 4-chloro-quinazolone-2-carboxylic acid, then cleavage via decarboxylation



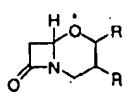
- Abell, C. [53]
- 1 ex; 64%
- condensation of resin-bound ethyl oxalate and 2-amino benzamide, then cleavage via decarboxylation



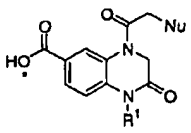
- R. W. Johnson [140]
- 35 ex; 46-98%
- from 4-fluoro-3-nitro-benzoic acid and β-amino esters



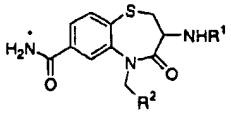
- Spyder [136]
- 380 members
- centrifuge based liquid removal



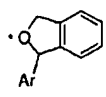
- Chmielewski, M. [73, 74]
- 2 ex; 26-30%
- intracyclative cationic cleavage



- Novo Nordisk [244]
- 6 ex; 26-43%
- from resin-bound 4-fluoro-3-*nitro*benzoic acid

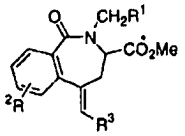


- Affymax [201]
- ca. 50 ex; ca. 50%
- S_NAr addition of cysteine to resin-bound 4-fluoro-3-nitrobenzoic acid

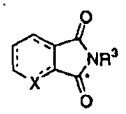


- Knochel, P. [193]
- 10 ex; 69-98%
- formation of resin-bound Grignard, addition of ArCHO, then intracyclative cleavage

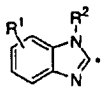
Table 9. (Continued)



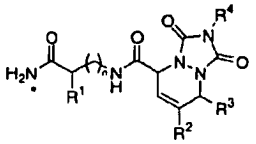
- Parke-Davis [25]
- ca. 8 ex; 34-73%
- ring formation via intramolecular Heck reaction



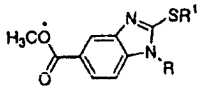
- Organon [12]
- 7 ex; 20-80%
- intracyclative cleavage of resin-bound 1,2-dicarboxylic acids



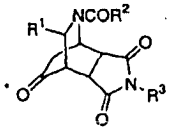
- COR Ther. [108]
- 9 ex; 85-90%
- TMOF/TFA-mediated cyclization of resin-bound anilino carbamates



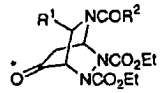
- Axys Pharm. [24]
- 10 ex; 24-82%
- [4+2] cycloaddition of resin-bound diene and urazine, then Mitsunobu to introduce R^4



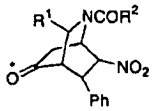
- Sun, C.-M. [241]
- 12 ex; 64-92%
- from resin-bound 4-fluoro-3-nitrobenzoic acid; soluble support



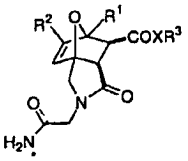
- SIBIA [46]
- 13 ex; 0-83%
- Diels-Alder reaction of resin-bound enol ether derived from N-acyl-2-substituted-dihydro-4-pyridone



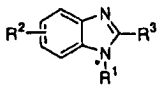
- SIBIA [46]
- 2 ex; 41-83%
- Diels-Alder reaction of resin-bound enol ether derived from N-acyl-2-substituted-dihydro-4-pyridine



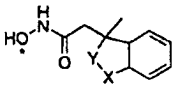
- SIBIA [46]
- 2 ex; 15%
- Diels-Alder reaction of resin-bound enol ether derived from N-acyl-2-substituted-dihydro-4-pyridine



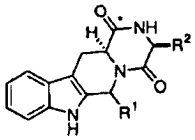
- Affymax [179]
- 14 ex; 83-98%
- intramolecular Diels-Alder reaction of resin-bound furans; X = O, NH



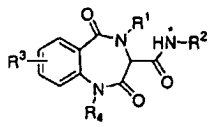
- SIDDCO [211]
- 10 ex; 30-74%
- reductive amination of resin-bound aldehyde, then N-arylation with *o*-fluoronitroaryls, NO_2 reduction, acylation, intracyclative cleavage



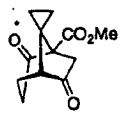
- Grigg, R. [82]
- 5 ex; 20-40%
- Pd-catalyzed intermolecular cascade reaction with aryl-iodides, CO, and resin-bound hydroxylamine



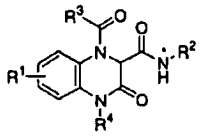
- Ganesan, A. [235]
- ca. 17 ex; 39-88%
- Pictet-Spengler condensation of N-acyliminium species prepared from resin-bound tryptophan, Fmoc-amino acid chlorides and RCHO, then Fmoc removal and intracyclative cleavage



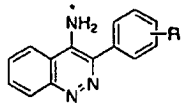
- RPR [109]
- 5 ex; 39-82%
- Ugi three-component condensation with ethyl-glyoxalate



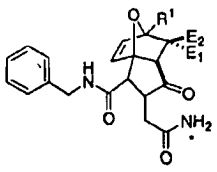
- Spitzner, D. [86]
- 1 ex; 79%
- resin-bound anionically-induced domino reactions; three derivatives also prepared



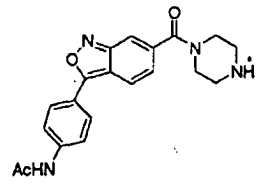
- RPR [109]
- 5 ex; 20-100%
- Ugi three-component condensation with ethyl-glyoxalate



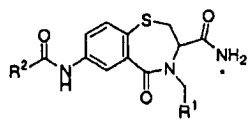
- Amgen [122]
- size and yield not given
- novel condensation from *o*-trifluorophenyl hydrazones



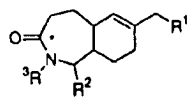
- Affymax [178]
- 6 ex; 88-95%
- tandem four-component condensation/intramolecular Diels-Alder reaction



- Novo Nordisk [216]
- 1 ex; 66%
- S_NAr displacement of 4-fluoro-3-nitrobenzoic acid amide with arylacetonitrile, then nitro reduction and cyclization

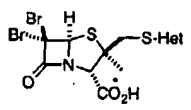


- Houghten, R. A. [163]
- ca. 4560 members
- from resin-bound cysteine and 2-fluoro-5-nitrobenzene carboxylic acid

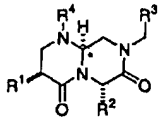


- Blechert, S. [200]
- 9 ex; 14-28%
- yne-ene cross metathesis and Diels-Alder cycloaddition then intracyclative cleavage

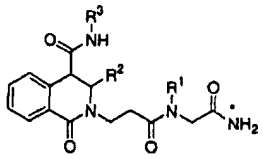
Table 9. (Continued)



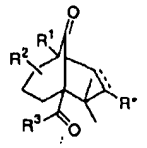
- Mata, E. G [62]
- 4 ex; 45-55%
- thermal rearrangement of penicillin sulfoxide



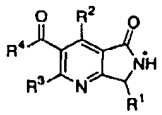
- Molecumetics [67]
- 11 ex; 22-71%
- acyliminium cyclization



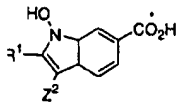
- Spyder [137]
- 30,816 members
- from resin-bound imine and homophthalic anhydride



- Nicolaou, K. C. [166]
- ca. 15 ex; 21-98%
- Se-mediated cyclization using polymer-supported reagent

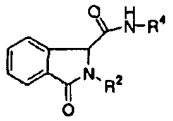


- Affymax [16]
- 4800 members
- sequential Hantzch condensation and intracyclative cleavage

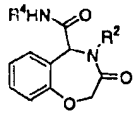


- Novo Nordisk [216]
- 3 ex; 39-74%
- S_NAr displacement of resin-bound 4-fluoro-3-nitrobenzoic acid with 1,3-dicarbonyls or acetonitriles, then nitro reduction and cyclization

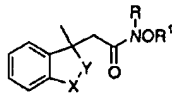
(b) Solution phase



- RepliGen [245]
- 13 ex; 75-100%
- Ugi three-component condensation



- RepliGen [245]
- 13 ex; 56-91%
- Ugi three-component condensation

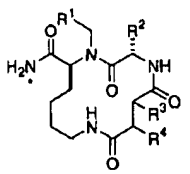


- Grigg, R. [82]
- 10 ex; 21-69%
- Pd-catalyzed intramolecular cascade reaction with *o*-iodo aryl ethers, CO, and protected hydroxylamines

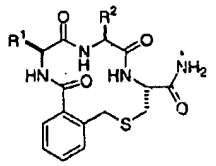
[a] Asterisk (*) indicates point of attachment to the resin.

Table 10. Polycyclic and Macrocyclic Synthesis: (a) Solid Phase, (b) Solution Phase[a]

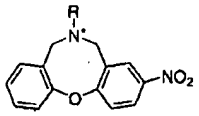
(a) Solid phase



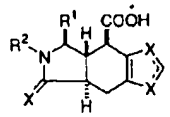
- SKB [133]
- 11 ex; 68-99%
- macrocyclization of lysine ε-NH_2 and succinamide carboxylic acid



- Burgess K. [69]
- 13 ex; 15-44%
- intramolecular cyclization of cysteine and benzyl-bromide

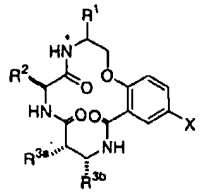


- Amgen [171]
- 5 ex; 38-53%
- reductive alkylation of resin-bound β-alanine with 2-fluoro-5-nitrobenzaldehyde and 2-hydroxybenzaldehyde, then intramolecular cyclization, cleavage, and N-alkylation

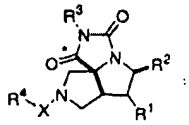


- R. W. Johnson [219]
- 2 ex; 36-37%
- intramolecular Diels-Alder reaction; X = CH, O; Y = O, 2H

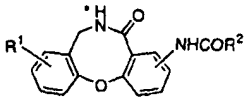
Table 10. (Continued)



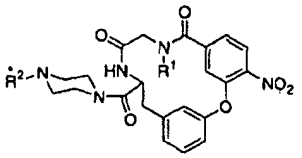
- Ibis Ther. [112]
- 26 ex; good purity
- Intramolecular ether formation via nucleophilic aromatic substitution; X = NO_2, NHR^4



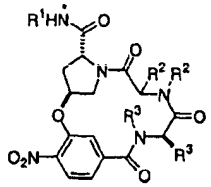
- Affymax [180]
- 12 ex; 14-34%
- Intramolecular azomethine ylide cycloaddition and intracyclative cleavage



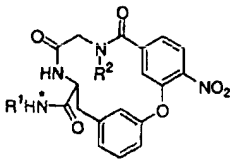
- Amgen [170, 172]
- 15 ex; 55-87%
- S_NAr cyclization from resin-bound phenols and 2-fluoro-5-nitrobenzoic acid



- Amgen [123]
- 20 ex; 52-68%
- S_NAr reaction

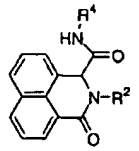


- Amgen [80]
- 18 ex; 55-78%
- S_NAr intramolecular cyclization of 4-OH-Pro

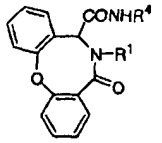


- Amgen [124]
- 30 members
- S_NAr cyclization

(b) Solution phase



- RepliGen [245]
- 13 ex; 38-64%
- Ugi three-component condensation



- RepliGen [245]
- 13 ex; 19-42%
- Ugi three-component condensation

[a] Asterisk (*) indicates point of attachment to the resin.

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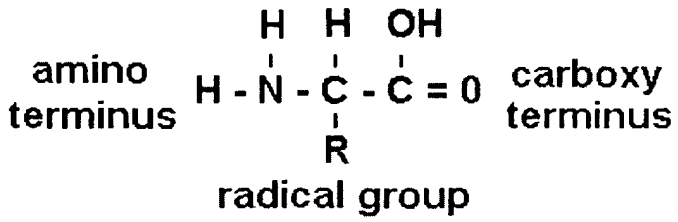
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Protein Structure & Function

In principle: Proteins are polymers of **amino acids**



[sometimes NH_3^+ & COO^- : depends on ***p*H**]

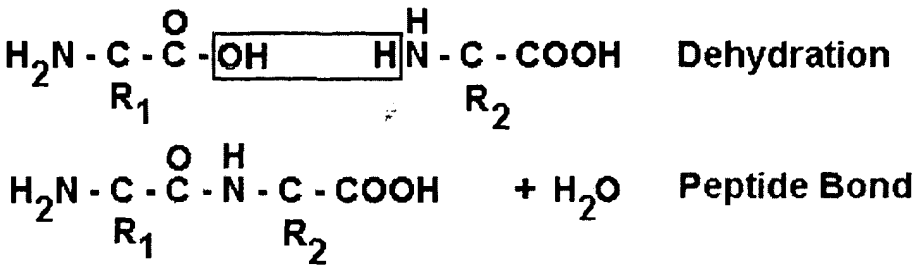
R = radical group:
asymmetric (**Homework**); levo ***(L)-rotatory*** [*cf.* dextro ***(D)-rotatory***]

determines chemical properties: **20 types** (note 3-letter & single-letter codes) (**MGA2_Table 3-1**)

Group properties	Three- & Single-letter codes
non-polar (**hydrophobic**)	ala, val, leu, ile, pro, met, phe, trp
	A V L I P M F W
polar (**hydrophilic**) (neutral charge)	gly, ser, thr, cys, tyr, asn, gln
	G S T C Y N Q
polar **basic** (**positive** charge)	lys, arg, his
	K R H
polar **acidic** (**negative** charge)	asp, glu
	D E

[Memorization of the abbreviations is *not* required for exams, but *will* make your lives as biologists easier!]

Dehydration of adjacent **carboxy** & **amino** termini forms **peptide bond** (**MGA2_03-21**)



Peptidyl Transferase catalyzes dehydration reaction:
carboxyl (**C**) terminus of growing **polypeptide** in **P** site
is cleaved from the **tRNA** &
joins to **amino** (**N**) terminus of new **amino acid** in **A** site

=> carboxyl end "***grows***": in the last example,

NH_2 - fmet - phe - gly - pro - COOH + NH_2 - lys - COOH

NH_2 - fmet - phe - gly - pro - lys - COOH

Recall that amino acid in **A** site is linked to **tRNA** through **COOH** terminus
Repeating, remnant backbone subunit **[N - C(R) - C]** is an **amino acid residue**

Proteins have four levels of structure

Primary Structure - order of amino acid residues in polypeptide (**MGA2_03-18a**) (Gr09-04a)

20^N possible, where **N** is number of residues
Potential for enormous variety:
e.g., $20^5 = 3.2 \times 10^6$ possible pentapeptides

Secondary Structure - configuration of **[-C-CN-]** backbone (**MGA2_03-18b**)
alpha helix: a right-handed helix
beta-pleated-sheet: parallel / antiparallel chains
both stabilized by **H-bonds**

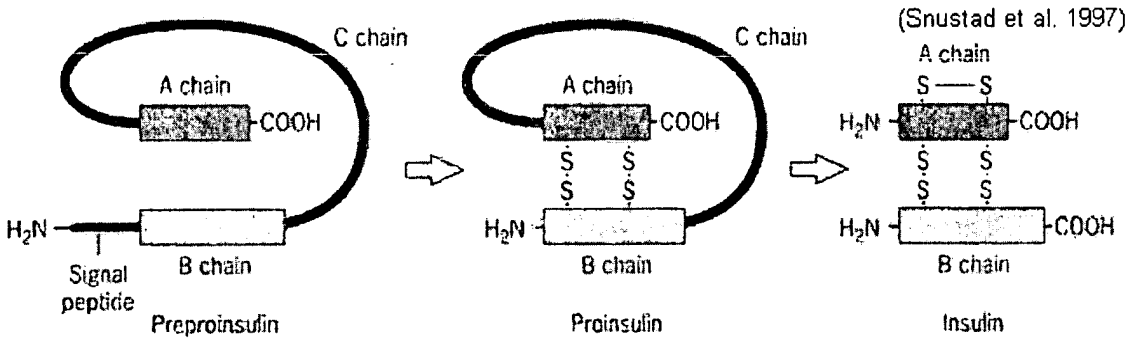
Tertiary Structure - 3-Dimensional folding of backbone (**MGA2_03-18c**)
cys + **cys** pairs form **disulfide bridges** (**- S - S -**)
pro residues form **hydrophobic "corners"**
hydrophilic residues occur on *exterior*,
participate in reactions in aqueous environments
hydrophobic residues occur in *interior*,
interact with membrane lipid bi-layer
gly fits in both hydrophobic & hydrophilic environments

Quaternary Structure - assembly of multiple subunits (**MGA2_03-18d**)
monomers / dimers / oligomers

e.g., hemoglobin is a **tetramer**: two alpha + two beta chains
charged residues (**asp, glu, lys, arg, his**) form ionic bonds bx subunits

Post-translational processing

Chemical modification of amino acids
addition of **formyl** group to **met** → **fmet**
Addition of **carbohydrate** side chains (**glycoproteins**)
ABO blood group proteins
Amino acids may be cleaved out of primary structure
e.g., biologically active **insulin** is less than half the primary sequence



preproinsulin → **proinsulin** → **insulin**
(110 aa's) (86 aa's) (51 aa's)

signal peptide (24 aa's) clipped from amino terminus
C peptide (31 aa's) excised from center
Tertiary (active) structure of insulin is
A chain (30 aa's) & **B chain** (21 aa's) held together by 3 disulfide bridges

Overview of protein function

Enzymatic catalysis of biological reactions
<> **Substrates** are bound in **active sites** (MGA2_03-36): the **Induced-Fit Model**
Lowered **energy of activation**
biological reactions occur at body temperature
with lower energy input
Anabolic - *synthesis* of complex molecules from simpler components
Ex., **transferases** synthesize peptide bonds
polymerases assemble nucleotides
Catabolic - *break-down* of complex molecules into simpler
Ex., **dehydrogenases** remove protons (H^+)
Amphibolic - *reversible* reactions
direction depends on relative concentration of precursor and product

Structural motifs recur in proteins with similar functions
Identification of motifs allows inferences about function
Helix - loop - helix motifs binds **Ca^{++}** (Gr09-10a)
Zinc - finger motifs (Gr09-10b) binds major & minor **DNA** grooves (Gr09-10c)

Other protein functions:
Structural:
Collagen constitutes 25% of human protein
Histones are the major components of **chromosomes**
[**online MGA animation** of **DNA** packing into chromosomes]

Transport:
Hemoglobin in blood & **myoglobin** in muscle bind **O_2**

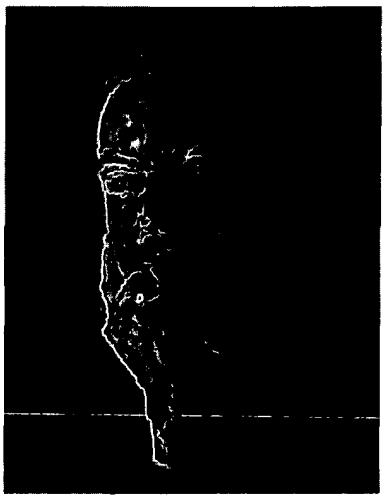
Miscellaneous:
immunoglobulins, hormones , etc.
Major Histocompatibility Complex (MHC) determines transplant success

***Drosophila* Genome Project** has cataloged >11,000 genes with protein products
2 / 3 have unknown functions (**MGA2, Table 3-5**)

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Home

Biology 2250 - Principles of Genetics



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Click on the following for:

Bio2250 Lab Manual v.04d (updated **28 Sept 2005**)
(**PDF format** requires **Acrobat reader**)
downloading requires ***password; see instructors***

Dr. Innes' Bio2250 webpage (Fall 2005)
Dr. Staveley's Bio2250 webpage (Fall 2001)

The Bio2250 Lecture & Lab schedule (updated 06 Sept 2005)
An orientation to the course (*please* read !!)
An introduction to the website
Tutorial on constructing webpages
Sample Quiz & Exam questions (updated 05 Dec 2005)

Link to Griffiths et al. (2003) Modern Genetic Analysis (2nd ed) online [MGA2]

See practice problems from Chaps 8, ,9 & 10 online

Some interesting Genetics links
Genetic Research in my lab

Bio2900 (Principles of Systematics & Evolution)	**(from Winter 2001)**
Bio4241 (Advanced Genetics)	**(next offered Fall 2006)**
Bio4505 (Biogeography & Systematics)	**(from Fall 2002)**
Bio4900 (Fundamentals of Geneic Biotechnology)	**(next offered Summer 2006)**
Med6390 (Human Population Genetics)	**(next offered Fall 2006)**

Activities of BIOS (MUN Biology Society)

Click here to e-mail me questions, comments, or suggestions.
Please include '2250' in the subject line

Lectures: TuTh 0900-1015 **Sn-2109** (Science Lecture Theatre)
Labs: MTuWTh 1400-1700 **Sn-4110** (Genetics & Evolution Laboratory)

Course lecture notes:

These notes are revised before & after lectures; check frequently for revisions.

	Topic	Last Revised	Lecture Date
	History of the Discovery of DNA	02 Aug 2005	- - - - - - - - - -
1	Structure of DNA: the Hereditary Molecule	02 Aug 2005	08 Sept 2005
2	How Genes Work I: DNA Replication & Transcription	02 Aug 2005	13 Sept 2005
3	The Genetic Code	02 Aug 2005	15 Sept 2005
4	How Genes Work II: RNA Translation	02 Aug 2005	20 Sept 2005
5	How Genes Work III: Protein Structure & Function Molecular Basis of Heredity	02 Aug 2005 29 Sept 2005	22 Sept 2005 27, 29 Sept 200
6	Molecular Basis of Mutation	29 Sept 2005	04 Oct 2005
	Midterm Exam	Sample	06 Oct 2005
	Dr Innes - Mendelian Genetics	Website	13 Oct - 17 Nov 2(
7	Genetic Engineering & Biotechnology	20 Nov 2005	22, 24 Nov 200
8	Applications of Biotechnology & Genomics	20 Nov 2005	20 Nov, 01 Dec 2(

ACKNOWLEDGMENTS - sources for images

Site last modified **05 December 2005**
This page has been accessed [—] times since **14 October 1997**

This page is dedicated to my daughter Jennifer Megan and my wife Justyna (MUN Research Report)

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HEMOGLOBIN S HAS AN ABNORMAL ELECTROPHORETIC MOBILITY

In 1949, Linus Pauling and his associates examined the physical-chemical properties of hemoglobin from normal people and from those with sickle-cell trait or sickle-cell anemia. Their experimental approach was to search for differences between these hemoglobins by electrophoresis. They found that the isoelectric point (p. 45) of sickle-cell hemoglobin is higher than that of normal hemoglobin in both the oxygenated and the deoxygenated state (Figure 7-42):

	Normal	*Sickle-cell anemia*	*Difference*
Oxyhemoglobin	6.87	7.09	0.22
Deoxyhemoglobin	6.68	6.91	0.23

These observations suggested that *there is a difference in the number or kind of ionizable groups in the two hemoglobins.* An estimate was made from the acid-base titration curve of hemoglobin in the neighborhood of pH 7. A change of one pH unit in the hemoglobin solution produces a change of about thirteen charges. The difference in isoelectric pH of 0.23 therefore corresponds to about three charges per hemoglobin molecule. It was concluded that *sickle-cell hemoglobin has between two and four more net positive charges per molecule than does normal hemoglobin.*

Patients with sickle-cell anemia (who are homozygous for the sickle gene) have hemoglobin S but no hemoglobin A. In contrast, people with sickle-cell trait (who are heterozygous for the sickle gene) have both kinds of hemoglobin in approximately equal amounts (Figure 7-43). Thus, Pauling's study revealed "*a clear case of a change produced in a protein molecule by an allelic change in a single gene.*" This was the first demonstration of a *molecular disease.*

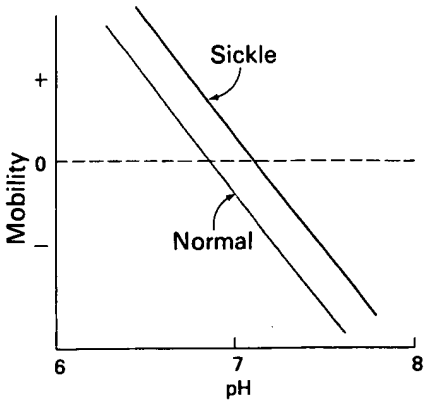


Figure 7-42
Electrophoretic mobility of sickle-cell hemoglobin and of normal hemoglobin as a function of pH. The isoelectric point of a molecule is the pH at which its mobility is 0.

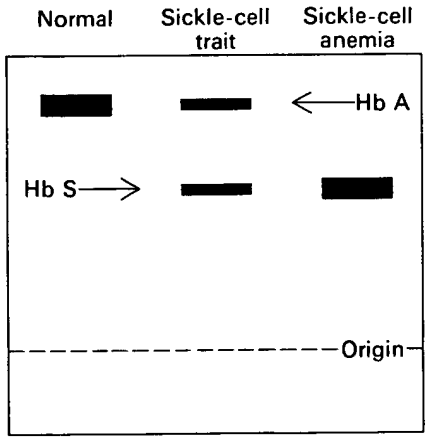


Figure 7-43
Gel electrophoresis pattern at pH 8.6 of hemoglobin isolated from a normal person, from a person with sickle-cell trait, and from a person with sickle-cell anemia.

A SINGLE AMINO ACID IN THE BETA CHAIN IS ALTERED IN SICKLE-CELL HEMOGLOBIN

The precise difference between normal and sickle-cell hemoglobin was identified in 1954, when Vernon Ingram devised a new technique for detecting amino acid substitutions in proteins. The hemoglobin molecule was split into smaller units for analysis, because it was anticipated that it would be easier to detect an altered amino acid in a peptide containing about 20 residues than in a protein ten times as large. Trypsin was used to specifically cleave hemoglobin on the carboxyl side of its lysine and arginine residues. Because an $\alpha\beta$ half of hemoglobin contains a total of 27 lysine and arginine residues, *tryptic digestion* produced 28 different peptides. The next step was to separate the peptides. This was accomplished by a *two-dimensional procedure* (Figure 7-44). The mix-

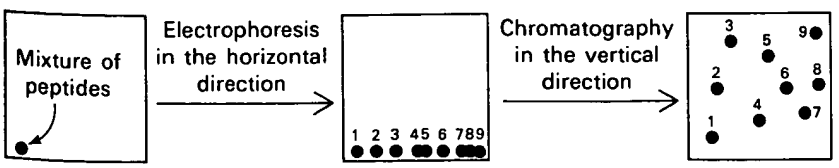


Figure 7-44
Fingerprinting. A mixture of peptides produced by proteolytic cleavage is resolved by electrophoresis in the horizontal direction followed by chromatography in the vertical direction.

Chromatography—
A term introduced by Mikhail Tswett in 1906 to describe the separation of a mixture of leaf pigments on a calcium carbonate column, like "light rays in the spectrum." Derived from the Greek words *chroma*, color, and *graphein*, to draw or write.

ture of peptides was placed in a spot at one corner of a large sheet of filter paper. *Electrophoresis* was first carried out in one direction, followed by *chromatography* in the perpendicular direction. Finally, the peptide spots were made visible by staining the filter paper with ninhydrin. This sequence of steps—selective cleavage of a protein into small peptides, followed by their separation in two dimensions—is called *fingerprinting*.

The fingerprints of hemoglobins A and S were highly revealing (Figure 7-45). When they were compared, *all but one of the peptide spots matched.* The one spot that was different was eluted from each fingerprint and shown to be a single peptide consisting of eight amino acids. Amino acid analysis indicated that this peptide in hemoglobin S differed from the one in hemoglobin A by a single amino acid. Ingram determined the sequence of this peptide and showed that *hemoglobin S contains valine instead of glutamate at position 6 of the β chain:*

Hemoglobin A	Val-His-Leu-Thr-Pro-Glu-Glu-Lys-
Hemoglobin S	Val-His-Leu-Thr-Pro-Val-Glu-Lys-
	β1 2 3 4 5 6 7 8

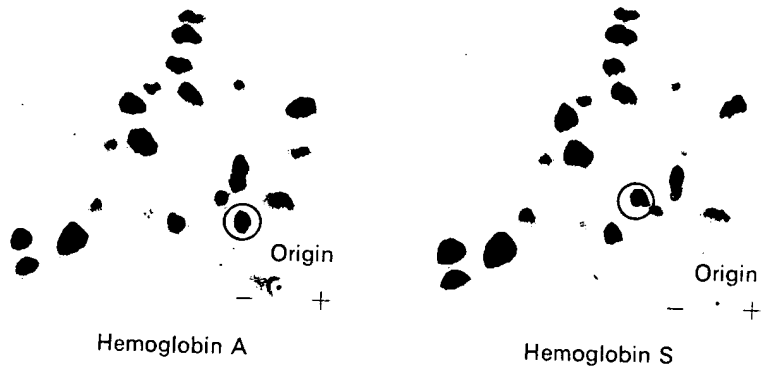


Figure 7-45
Comparison of the ninhydrin-stained fingerprints of hemoglobin A and hemoglobin S. The position of the peptide that is different in these hemoglobins is encircled in red. [Courtesy of Dr. Corrado Baglioni.]

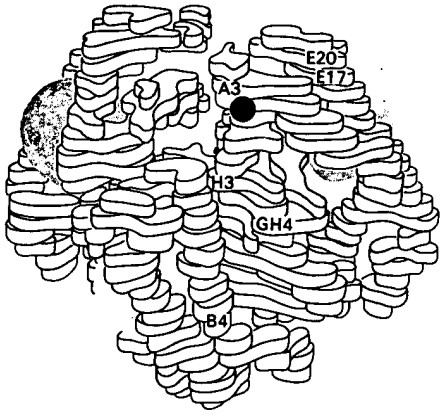


Figure 7-46
The position of the amino acid change in hemoglobin S (glutamate to valine at β6) is marked in red in this model of deoxyhemoglobin. Note that this site is at the surface of the protein. The α chains are shown in yellow, the β chains in blue. [After J. T. Finch, M. F. Perutz, J. F. Bertles, and J. Dobler. *Proc. Nat. Acad. Sci.* 70(1973):721.]

SICKLE HEMOGLOBIN HAS STICKY PATCHES ON ITS SURFACE

The side chain of valine is distinctly nonpolar, whereas that of glutamate is highly polar. The substitution of valine for glutamate at position 6 of the β chains places a nonpolar residue on the outside of hemoglobin S (Figure 7-46). *This alteration markedly reduces the solubility of deoxygenated hemoglobin S but has little effect on the solubility of oxygenated hemoglobin S.* This fact is crucial to an understanding of the clinical picture of sickle-cell anemia and sickle-cell trait.

The molecular basis of sickling can be visualized as follows:

1. The substitution of valine for glutamic acid gives hemoglobin S a sticky patch on the outside of each of its β chains (Figure 7-47). This sticky patch is present on both oxy- and deoxyhemoglobin S and is missing from hemoglobin A.

2. In the EF corner of each β chain of deoxyhemoglobin S is a hydrophobic site that is complementary to the sticky patch (Figure 7-47). The complementary site on one deoxyhemoglobin S molecule can bind to the sticky patch on another deoxyhemoglobin S molecule, which results in the formation of long fibers that distort the red cell.

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Transforming Growth Factor α: Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities

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Received 22 July 1987/Accepted 30 November 1987

To study the relationship between the primary structure of transforming growth factor α (TGF-α) and some of its functional properties (competition with epidermal growth factor (EGF) for binding to the EGF receptor and induction of anchorage-independent growth), we introduced single amino acid mutations into the sequence for the fully processed, 50-amino-acid human TGF-α. The wild-type and mutant proteins were expressed in a vector by using a yeast α mating pheromone promoter. Mutations of two amino acids that are conserved in the family of the EGF-like peptides and are located in the carboxy-terminal part of TGF-α resulted in different biological effects. When aspartic acid 47 was mutated to alanine or asparagine, biological activity was retained; in contrast, substitutions of this residue with serine or glutamic acid generated mutants with reduced binding and colony-forming capacities. When leucine 48 was mutated to alanine, a complete loss of binding and colony-forming abilities resulted; mutation of leucine 48 to isoleucine or methionine resulted in very low activities. Our data suggest that these two adjacent conserved amino acids in positions 47 and 48 play different roles in defining the structure and/or biological activity of TGF-α and that the carboxy terminus of TGF-α is involved in interactions with cellular TGF-α receptors. The side chain of leucine 48 appears to be crucial either indirectly in determining the biologically active conformation of TGF-α or directly in the molecular recognition of TGF-α by its receptor.

Transforming growth factor α (TGF-α) is a polypeptide of 50 amino acids. First isolated from a retrovirus-transformed mouse cell line (9), it has subsequently been found in human tumor cells (10, 29), in the early rat embryo (18), and recently in cell cultures from the pituitary gland (23). TGF-α appears to be closely related to epidermal growth factor (EGF) structurally and functionally (19, 20). The two peptides apparently bind to the same receptor, and both induce anchorage-independent growth of certain nontransformed cells, such as NRK cells, in the presence of TGF-β (1).

Comparison of amino acid sequences reveals about 35% homology among the EGF-like peptides (rat [27], mouse [25], and human [13] EGFs and rat [19] and human [12] TGF-αs). Some viral peptides (Shope fibroma growth factor [6], vaccinia growth factor [2], and myxoma growth factor [30]) also share homologies with the EGF-like peptides.

If TGF-α is involved in transformation, a TGF-α antagonist could be an important therapeutic tool in the treatment of certain types of malignancies. An understanding of the conformational and dynamic properties of the TGF-α molecule is basic to the design of an antagonist. A hypothetical antagonist would bind to the same receptor as TGF-α, but would not induce the series of proliferative and transforming events induced by TGF-α. To obtain such a molecule it is necessary to dissociate interactions responsible for binding from those involved in signal transduction. We decided to approach the problem by way of site-directed mutagenesis of a human sequence of TGF-α. In this report we describe our first series of mutations, which were carried out at residues Asp-47 and Leu-48, in the carboxy-terminal part of TGF-α; these two amino acids are highly conserved in the EGF-like family of peptides. We show that these two adjacent residues play different roles in the structure and/or function of TGF-α.

MATERIALS AND METHODS

Cells. Normal rat kidney (NRK) cells were grown in Dulbecco modified Eagle medium containing 10% (vol/vol) calf serum.

TGF-α gene. The sequence of the 50-amino-acid human TGF-α was originally derived from a human TGF-α precursor cDNA (12). The coding sequence is preceded by an ATG methionine codon and followed by a TAA stop codon and is flanked by *Eco*RI restriction sites. This *Eco*RI fragment combines the 59-base-pair *Eco*RI-*Nco*I fragment from plasmid pTE5 (12) with the 111-base-pair *Nco*I-*Eco*RI fragment from plasmid pyTE2 (11). The resulting *Eco*RI fragment was inserted in M13mp18 for site-directed mutagenesis.

Synthesis and purification of oligonucleotides and oligonucleotide-directed mutagenesis. The synthesis and purification of 20- to 27-nucleotide oligonucleotides were carried out as described previously (31). The one or two nucleotides responsible for the mutation were located in the middle of the oligonucleotide. Mutagenesis was performed by published procedures (21, 33). The sequences of the mutant clones were verified by the method of Sanger et al. (25).

Yeast shuttle vector. The vector YEp70αT contains a yeast α-factor pheromone promoter and prepro sequence for the expression of TGF-α (15). The mutant TGF-α coding sequence was inserted in the *Eco*RI site of plasmid YEp70αT and expressed in the form of a fusion protein consisting of 92 amino acids from the prepro sequence of the yeast α factor attached to the amino terminus of TGF-α (28). The yeast cleaves the precursor and secretes TGF-α with 8 amino acids fused to it (4 are encoded by the prepro sequence of α-factor, and the other 4 are encoded by the DNA sequence added to insert of the TGF-α gene). The last of these residues is a methionine, which allows the cleavage of the secreted fusion

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protein by cyanogen bromide (CNBr) and the release of a mature TGF-α (50 amino acids) (see Results).

Yeast strain and transformation. The yeast *Saccharomyces cerevisiae* 20B-12 (*MATα trp1 pep4-3*) (17) was obtained from the Yeast Genetics Stock Center, Berkeley, Calif. *S. cerevisiae* 20B-12 was grown in YEPD medium (1% yeast extract [Difco Laboratories], 2% Bacto-Peptone [Difco], 2% glucose). When the culture reached an optical density at 660 nm of 1, spheroplasts were prepared (14) for transformation. For each transformation we used 10 to 15 μg of purified plasmid DNA.

Partial purification of TGF-α mutants. At 3 days after transformation, five individual colonies of transformants were grown to saturation in YEPD medium. The amount of protein in the yeast medium was measured by the method of Bradford (3), and the amount of mutant TGF-α secreted in the yeast medium was determined by radioimmunoassay. The clones which secrete the highest amount of mutant TGF-α were used to grow a 1-liter culture in YNB-CAA medium (0.67% yeast nitrogen base, 20 g of glucose per liter, 10 g of Casamino Acids [Difco] per liter). After the culture reached saturation (optical density at 660 nm of 10 to 12) (48 h in an air shaker at 30°C), the yeast conditioned medium was dialyzed extensively against 1 M acetic acid in 3,000-molecular-weight cutoff dialysis tubing. Usually 250 ml of dialyzed culture was lyophilized, suspended in 10 ml of 70% formic acid, and treated with CNBr (molar excess of 500) for 20 h at room temperature. The CNBr was subsequently evaporated, and the samples were lyophilized. CNBr-treated samples were suspended in 1 ml of 1 M acetic acid, loaded on a Bio-gel P30 column (30 by 1.5 cm [Bio-Rad Laboratories]), and eluted with 1 M acetic acid. Fractions of 1 ml were collected. Aliquots were lyophilized, suspended in binding buffer (minimum essential medium containing 1 mg of bovine serum albumin per ml and 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4]), neutralized if necessary to pH 7.4, and tested in EGF-binding competition and soft-agar assays, as well in radioimmunoassay.

Radioimmunoassays. The amounts of TGF-α secreted in the yeast medium were determined by radioimmunoassay with the immunoglobulin G fraction of a polyclonal antibody, 34D, raised against recombinant human TGF-α (4), in 0.1 M Tris (pH 7.5)–0.15 M NaCl–2.5 mg of bovine serum albumin per ml. The amounts of partially purified TGF-α present in the P30 column fractions were measured by using the Biotope RIA kit with polyclonal antibody against human TGF-α (a gift from W. Hargreaves, Biotope), under denaturing conditions, as recommended by the supplier.

EGF binding competition assay and soft agar assay. Both EGF-binding competition and soft-agar assays have been described previously (1).

RESULTS

Rationale for mutations in the carboxyl terminus of TGF-α. Figure 1 shows the amino acid sequence of TGF-α in which the residues that are conserved among all the EGF-like peptides described thus far (EGF, TGF-α, and EGF-like viral proteins) are enclosed in bold circles. Among the 11 conserved amino acids, there are 6 Cys and 2 Gly residues, which presumably play essential roles in determining the overall conformation of the molecule. We concentrated on the two conserved amino acids in the carboxyl terminus, Asp-47 and Leu-48. The Asp in position 47 is conserved among the EGFs and TGF-α (human or murine), but not

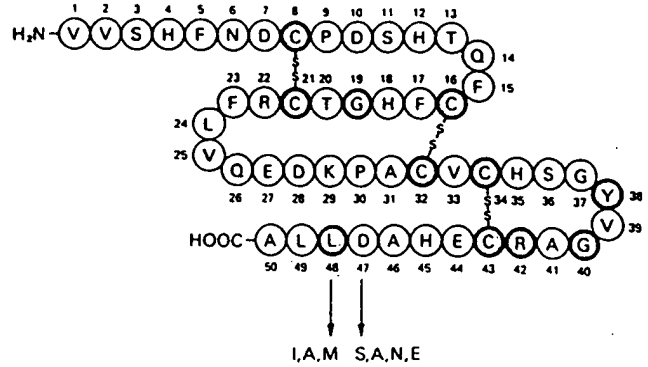


FIG. 1. Mutations in the carboxy terminus of human TGF-α. The amino acids conserved in all the family of EGF-like growth factors (human and murine EGFs and TGFs, as well as the gene products of the vaccinia virus [vaccinia growth factor], the Shope fibroma virus [Shope fibroma growth factor], and the myxoma virus [myxoma growth factor]) are enclosed in bold circles. The mutations of amino acids at positions 47 and 48 are indicated. Symbols: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

among the EGF-like viral proteins (vaccinia growth factor, Shope fibroma growth factor, or myxoma growth factor), whereas Leu 48 is conserved among all the EGF-like peptides so far described. In both mouse and human EGF, the two corresponding residues (Asp-46 and Leu-47) are located near the surface of the protein (8, 22, 22a). We designed a series of mutations in these two positions.

Asp-47 has been mutated to Glu, Asn, Ser, and Ala. Glu was chosen because it has the same charge as and a larger size than Asp; Asn has a similar side-chain structure, but is uncharged; Ser is smaller but still polar; Ala is smaller and nonpolar.

Leu 48 has been mutated to Ile and Met, which are both large, nonpolar residues like Leu, and to Ala, which is nonpolar but smaller. We introduced the chosen mutations by site-directed mutagenesis of the cloned human TGF-α gene, using synthetic oligonucleotides.

Construction of the yeast α mating pheromone–human TGF-α plasmid. The TGF-α expression vector pyTE1 (Fig. 2) was constructed by using plasmid YEp70αT (15) which contains the 2μm origin of replication and yeast *TRP1* gene for its replication and selective maintenance, respectively. YEp70αT also contains the yeast α-factor promoter, the α-factor prepro sequence coding for 89 amino acids, and the sequence for 3 amino acids resulting from the introduction of *Xba*I and *Eco*RI sites. The human mature TGF-α sequence (12) is contained in a 170-base-pair *Eco*RI fragment which includes an ATG (Met) codon preceding the sequence of TGF-α and a TAA (stop) codon followed by 8 nucleotides. This TGF-α sequence was inserted in the unique *Eco*RI site of YEp70αT. Clones with the proper orientation were selected, and DNA was isolated for yeast transformation.

Measurement of TGF-α secreted by *S. cerevisiae*. The amount of total proteins secreted into the yeast culture was 10 ± 1 μg/ml for wild-type as well as mutant TGF-α as determined by the method of Bradford (3). Before further purification was attempted, we wanted to determine whether the mutated TGF-α proteins were being secreted by the yeast. The low pH of the yeast medium, as well as the acidic proteins secreted in the yeast culture, precluded biological assay of secreted mutants. Therefore, immunological meth-

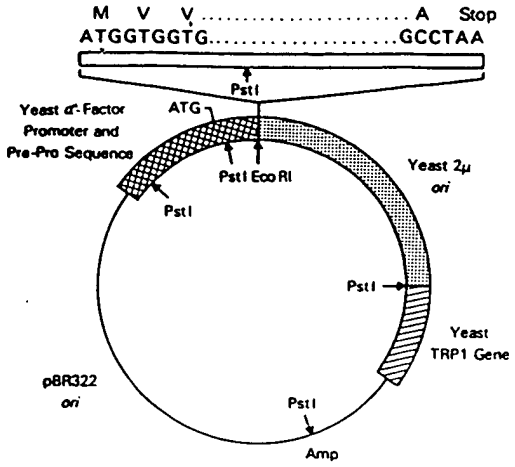


FIG. 2. Structure of the *S. cerevisiae* 8.2-kilobase shuttle vector vTE1. The secretion of the TGF-α gene is under the transcriptional ntrol of the yeast α-factor promoter and prepro sequence (▨). ie yeast 2μm origin of replication (▩) and the selective yeast *RP1* gene (▧) are indicated. The TGF-α gene, preceded by an itiation (ATG) codon and followed by a stop (TAA) codon, is serted in the *Eco*RI site. Details are given in Materials and lethods and in Results.

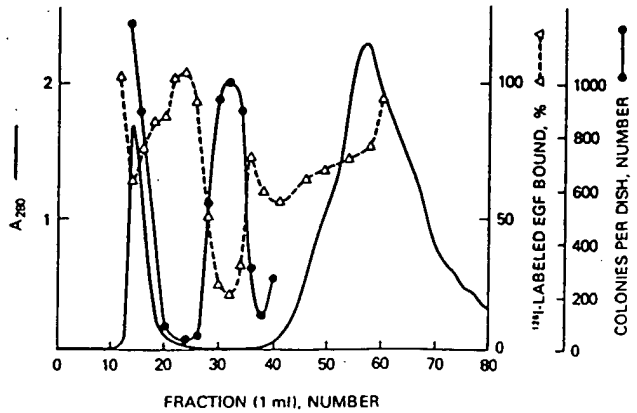


FIG. 3. Purification of yeast-secreted wild-type TGF-α. The purification procedure is described in Materials and Methods and in Results. Aliquots of every other fraction of the Bio-Gel P30 column were tested for their abilities to compete with ^{125}I-EGF for binding to the EGF receptor (△) and to induce colony formation (>62 μm) on NRK cells in soft agar in the presence of TGF-β (1 ng/ml) (●). The A_{280} profile of the proteins was determined (——).

ds were used. Wild-type and mutant TGF-α's were se-eted at a level of 100 to 200 ng/ml and 10 to 500 ng/ml, spectively (as determined by radioimmunoassay with poly-onal antibody 34D). We thus estimate that the percentage TGF-α secreted in the yeast culture is at least 1% of the tal protein secreted. We cannot yet assess whether the riations in the levels of secretion of different mutant GF-α proteins are real or whether one single-amino-acid bstitution drastically affects the recognition by the anti-dy. The latter hypothesis is the more likely, since the use another polyclonal antibody (Biotope) under denaturing nditions enabled us to detect certain TGF-α mutants (such [Ala 47]-TGF-α, in which the amino acid in position 47 of man TGF-α is mutated to an alanine) that were poorly tected by 34D, under nondenaturing as well as denaturing nditions. After the amount of TGF-α mutant proteins was timated, the medium was extensively dialyzed against 1 M etic acid and lyophilized as described in Materials and ethods.

Partial purification of yeast-secreted TGF-α. Although the ast shuttle vector was constructed in such a way as to crete TGF-α with 8 amino acids fused to the N terminus, was often observed that a significant fraction of the creted TGF-α was in a higher-molecular-weight fragment rresponding to the size expected from an uncleaved (un-ocessed) 92-amino-acid fusion protein. Since a Met had en introduced at the N terminus of TGF-α and since GF-α contains no Met in its sequence, CNBr treatment uld be used to cleave either of these 8- or 92-amino-acid terminal peptides and release the complete 50-amino-acid GF-α. Indeed, CNBr treatment of yeast-secreted proteins sulted in the conversion of high-molecular-weight TGF-α o the 6,000-molecular-weight species, as revealed by estern immunoblot (data not shown).

CNBr-cleaved samples (see Materials and Methods) were rified on a Bio-Gel P30 column. Figure 3 shows the elution ofile of the proteins, as well as the results of a radioreceptor assay and a soft-agar assay performed on aliquots of the umn fractions. The A_{280} profile shows two major peaks of eluted proteins, one corresponding to the void volume and the other one to proteins of molecular weight <3,000. Aliquots of the column fractions were tested for their ability to compete with ^{125}I-EGF for binding to the receptor. The fractions that were the most active in this assay were located between the two major protein peaks, in an area where relatively few proteins eluted. Although some activity was found in the first protein peak (void volume), this was considerably reduced on treatment with stronger CNBr (data not shown).

Aliquots of each fraction were also tested for their ability to induce anchorage-independent growth of NRK cells in soft agar in the presence of TGF-β (1 ng/ml). The receptor binding and colony-forming activity superimposed almost exactly (Fig. 3). Analysis by polyacrylamide gel electrophoresis with silver staining, as well as by Western blot, of the column fractions shows that our purification procedure (CNBr cleavage followed by P30 sizing column) eliminates high-molecular-weight proteins (data not shown). Since pure TGF-α migrates in a broad band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (32), this technique cannot be used for proper assessment of the degree of separation of TGF-α from low-molecular-weight contaminating proteins. Nevertheless, within our detection levels the amounts of TGF-α present in the column fractions (detected by radioimmunoassay using the antibody from Biotope) correlated with the amounts observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown).

Comparison of binding and colony-forming activity of TGF-α partially purified from yeast media. It was important to show that wild-type TGF-α secreted from *S. cerevisiae* had the expected biological properties and that its activity in soft-agar and radioreceptor assays was equivalent. For these assays, the amount of EGF-competing activity present in the most active fraction of the P30 column of wild-type TGF-α was measured in terms of EGF equivalents. The dilution curve had a slope that was parallel to that of the EGF standard. This value was also used to measure the colony-forming activity of the partially purified wild-type TGF-α (with EGF as a standard in the assay). The colony-forming activity of the partially purified wild-type TGF-α corre-

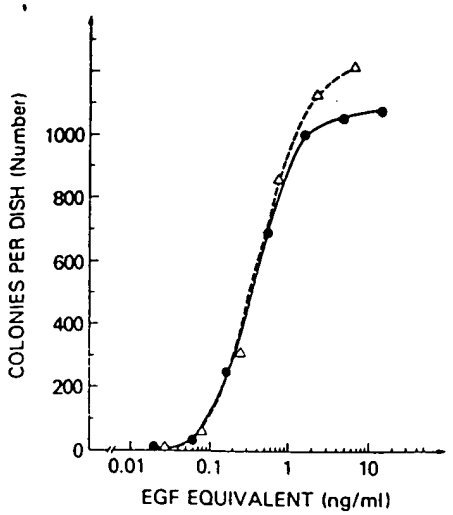


FIG. 4. Correlation between the activities in the binding and colony-forming assay for the partially purified wild-type TGF-α secreted by *S. cerevisiae*. The activity in the radioreceptor assay of the peak fraction from the P30 column was determined in EGF equivalent concentration. The value obtained was used for the soft-agar assay. Colonies of >62 μm (Δ) and the EGF standard (●) are shown.

sponded exactly to that of EGF (Fig. 4). Thus, we have partially purified a wild-type 50-amino-acid TGF-α showing the expected binding and colony-forming activities, which provides a reference substance for mutant TGF-αs that might show a dissociation of binding and colony-forming abilities.

Biological and biochemical activities of the partially purified TGF-α mutant proteins. Mutated TGF-αs were expressed by using the yeast system and partially purified on Bio-Gel P30 columns as described in Materials and Methods. Mutant TGF-αs were usually obtained from two different clones of yeast transformants. The CNBr-cleaved samples were purified through different Bio-Gel P30 columns for each mutant protein to avoid any possible contamination from one peptide to another. The purification profiles observed with the mutant TGF-αs were similar to those obtained for the wild-type TGF-α. Aliquots of the P30 column fractions were tested in radioreceptor and soft-agar assays. For all mutant proteins, the highest activity in both assays was always found in the same fraction of the Bio-Gel P30 column effluent (peak fraction). Extensive purification of a series of mutant proteins for screening purposes is not practical. Therefore, we needed a quantitation system that would allow us to compare mutant proteins with each other. Thus, the amount of TGF-α present in the peak fraction was estimated by radioimmunoassay with an antiserum to native TGF-α (obtained from W. Hargreaves), under denaturing conditions, as described in Materials and Methods. All values given in Table 1 were obtained from the peak fraction.

The controls done with the wild-type TGF-α showed (Fig. 4; Table 1) that binding and transforming activity were equivalent. The yeast vector without a TGF-α insert did not secrete any EGF-like proteins, as determined by both radioreceptor and soft-agar assay.

Two types of results were obtained upon assay of mutant proteins having different amino acid substitutions at Asp-47. In both [Ala-47]–TGF-α and [Asn-47]–TGF-α, binding ability was retained. Soft-agar and radioreceptor activities correlated for [Asn-47]–TGF-α; there was a lower value for

TABLE 1. Biological and biochemical activities of mutant TGF-α proteins secreted by *S. cerevisiae* and partially purified

Insert in the yeast expression vector	EGF equivalence (ng/ml) in:		Amt of TGF-α (ng/ml) in radioimmunoassay
	Radioreceptor assay	Soft-agar assay	
Wild-type TGF-α	700	700	2,000
	400	300	ND[a]
None	0	0	0
[Ala-47]–TGF-α	100	44	220
	66	48	ND
[Asn-47]–TGF-α	80	72	180
	75	72	525
[Glu-47]–TGF-α	3	3	42
[Ser-47]–TGF-α	10	4	60
[Ala-48]–TGF-α	0	0	16
	0	0	220
[Ile-48]–TGF-α	4	12	470
	2	7	490
[Met-48]–TGF-α	2	8	453
	0.5	2	420

[a] ND, Not determined.

colony-forming activity than for EGF-binding competition for [Ala-47]–TGF-α. [Ser-47]–TGF-α and [Glu-47]–TGF-α appeared to have lower activities in both assays than either wild-type TGF-α or [Ala-47]–TGF-α and [Asn-47]–TGF-α. These results indicate that neither the carboxyl charge nor the polarity of Asp-47 is essential for biological activity.

The effects of mutation of Leu-48, one of the 11 amino acids perfectly conserved among all the EGFs, TGF-αs, and viral EGF-like proteins, are dramatic. [Ala-48]–TGF-α totally lacked binding and colony-forming activity. [Ile-48]–TGF-α and [Met-48]–TGF-α had very little biological activity compared with wild-type TGF-α. Another substitution, [Met-48]–TGF-α, resulted in a truncated mutant lacking the last 2 amino acids and having a substitution of Leu to homoserine at position 48 following treatment with CNBr. Alternatively, if [Met-48]–TGF-α was not treated with CNBr, fusion proteins of TGF-α (mutated to Met in position 48) with 8 or 92 amino acids attached at the N terminus were obtained. Very low activities in binding and soft-agar assays were found for these mutants, whether or not they were cleaved with CNBr. Experiments on EGF and TGF-α have shown that an N-terminal extension does not markedly modify EGF-binding activity (12, 26). Therefore, the loss of activity obtained with [Met-48]–TGF-α that has not been CNBr treated was probably due to the mutation itself and not to the N-terminally extended fusion protein. We do not know whether the loss of activity observed with the TGF-α shortened to 48 amino acids and having a substitution of Leu-48 to homoserine is due only to the mutation or also to the lack of the last 2 amino acids.

The data obtained by radioimmunoassay on the partially purified wild-type and mutant TGF-α show that the amount of TGF-α detected was always higher than the amount determined by measurement of biological activity. This may be due to the presence in the fraction of a certain percentage of incorrectly folded TGF-α that might be recognized in a

)immunoassay under denaturing conditions but would be biologically active. None of the mutant proteins ied to be present in amounts equivalent to those ob- ed for wild-type TGF-α in the partially purified fractions ther radioimmunoassay, radioreceptor, or soft-agar as- was used for quantitation). It is not clear whether istently less TGF-α was produced by the mutant con- ts than by the wild type or whether the secreted mutant ins were simply less well recognized by the antibody. use of these uncertainties, the biological activities of lifferent mutant proteins cannot be accurately related to own amount of mutant TGF-α protein. Even though)immunoassay should be used with caution for a quan- ve evaluation of mutant TGF-α proteins, a positive tion demonstrates that immunoreactive TGF-α was ent in the P30 peak fraction for each mutant. Therefore, act that one of the mutant proteins ([Ala-48]–TGF-α) is)gically inactive can be attributed to the mutation itself, not to the lack of production of the mutant protein by the t or its loss through purification. However, if the mutant ins are in fact as immunoreactive as the wild type, then -47]–TGF-α and [Asn-47]–TGF-α are as active as wild- TGF-α and [Glu-47]–TGF-α and [Ser-47]–TGF-α are active; in contrast, [Ile-48]–TGF-α and [Met-48]–TGF-α almost inactive. The differences between mutation of -47 and Leu-48 would then be even more striking.

DISCUSSION

GF-α shows sequence homologies with EGF, and both th factors share the same cellular receptors (20). Even gh EGF was discovered 25 years ago (7) and its prop- s have been extensively studied over the years (5), the ing site of EGF to its receptor has still not been rmined, and the relationship between structure and tion of EGF/TGF-α is still to be discovered. Particu- , we do not know whether binding to the receptor and al transduction occur through one or more domains of nolecule or through which amino acids. We approached question by performing site-directed mutagenesis of -α and focused our attention on two adjacent amino s, Asp-47 and Leu-48, located in the carboxy terminus highly conserved in the EGF-like family of peptides. xpectedly, these two amino acids showed very different itivities to mutation and particularly to a substitution to [Ala-47]–TGF-α retained binding and colony-forming ities, whereas [Ala-48]–TGF-α completely lost both ities. These data show that Asp-47 and Leu-48 play very rent roles in defining the structure and/or the activity of -α. The other mutations performed on Asp-47 were titutions to Asn, Ser, and Glu. [Asn-47]–TGF-α, like -47]–TGF-α, was active in binding and induction of ny formation, but [Ser-47]–TGF-α and [Glu-47]–TGF-α ved weaker growth factor activities. These results indi- that neither the carboxyl charge nor the polarity of -47 is essential for biological activity. Interestingly, two ie EGF-like viral proteins, myxoma growth factor and e fibroma growth factor (6, 30), have Asn instead of Asp)sition 47; we have shown that [Asn-47]–TGF-α retains)gical activity.

ibstitution of Leu-48 to Met and Ile led to mutant eins with very low activities, whereas substitution to Ala o complete loss of activity. We did not expect that a ution of Leu to Ile (which have similar sizes and polari- would cause such a strong effect. Thus, Leu-48, which)nserved perfectly among all the EGF-like peptides, seems to be essential, through its exact geometry, for the biological activity of TGF-α.

The mutant proteins tested so far, when active, showed parallel behaviors in binding and colony formation. Some mutant proteins lost all activities, and we assume that the binding capacity has been lost. We have not been able to dissociate the binding and colony-forming abilities by using any of the present series of mutant proteins, and it is necessary to screen more of them in search of an antagonist of TGF-α.

Results relating to the biological activity of EGF show that derivatives of mouse EGF and human EGF (EGF 1-47) lacking the carboxy-terminal 6 amino acids as a result of enzymatic digestion are less potent than the intact molecule in mitogenic stimulation of fibroblasts, but retain full biological activity in in vivo assays (inhibition of gastric acid secretion) (16). On the other hand, naturally occurring truncated forms of rat EGF, which lack the carboxy-terminal 5 amino acids (rEGF 2-48) are as potent as mouse EGF (mEGF 1-53) in receptor-binding and mitogenic assays (27). We do not know whether the discrepancies observed are due to the origin of the molecule (artificial or natural) or to the type of bioassay used. In any event, all of these EGF-related molecules, which are shorter than mouse or human EGF, still retain Leu-47. We have shown that in TGF-α, the corresponding residue, Leu-48, is critical for the biological activity.

Recent data on the three-dimensional structure of mouse EGF obtained by nuclear magnetic resonance show that even though Asp-46 and Leu-47 (Asp-47 and Leu-48 in TGF-α) are both solvent accessible (8, 22, 22a), their side chains point in opposite directions in the beta-sheet structure. Therefore, the role of these adjacent amino acids in the structure and, consequently, the function of EGF might be very different. Our data show that the amino acids Asp-47 and Leu-48 of TGF-α are not equally important for the biological activity of TGF-α, despite their conservation among the EGF-like peptides. From the dramatic loss in biological activity which is characteristic of mutation of Leu-48, we also suggest that this residue is involved in binding to the cellular receptors either by direct interaction with the receptor or by providing the proper conformation to the molecule.

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APPENDIX 1

Test Substance Sources

I. PURE COMPOUNDS

Aldrich Chemical Company
An early resource in the supply of test compounds, Aldrich drew upon the 30,000+ compounds in its Alfred Bader collection. This collection, which predated the demand by several years, was accumulated from purchases of academic collections of emminent chemists and other diverse sources. Constitutents were first available individually at a nominal charge. Today the collection has grown significantly with pricing competitive with standard brokers. Aldrich also offers their compounds in microplates. Information: Tel: 1-800-558-9160.

Bionet Research Limited (UK)
Bionet offers a library of >10,000 synthetic compounds which are derived directly from conventional synthesis conducted by Bionet or its affiliate Key Organics Ltd. Rapid return is promised and delivered in follow-up supplies and scale-up. Their collection includes multiple analogs of aryl and heterocyclic core structures. Cost is based on quantity purchased. Electronic data files of their collection are availaable without charge. Information: Colin Deane, Bionet Research Limited, 3 Highfield Industrial Estate, Camelford, Cornwall PL32 9QZ. UK. Tel: 44-1840-212171; Fax: 44-1840-213712.

BioScreen International, Inc. (USA)
BioScreen International Inc. has access to thousands of new chemical entities with undeveloped potential for different applications. Such compounds are from reliable new and established sources in Eastern Europe and the former Soviet Union. The up-to-date lists of available compounds can be provided for selection in either hard copy or computer readable format (MACCS or ChemBase). Selection of the compounds may be truly random or based on designated chemical structure categories of interest to the client. Compounds of designated chemical structures may also be custom synthesized. Various quantities are available, including, but not limited to 1-5 mg, 50-100 mgs, or gram quantities. Information is provided in form of floppy disks or printed lists. Lists contain (if available) structure, chemical name(s), and all known relevant data. Compounds of interest are selected by reference number. Compounds may be purchased on a non-exclusive or exclusive basis. The price per selected compound is generally $80/100 mg sample but varies according to quantity ordered, amount, exclusivity vs. nonexclusivity, and requested criteria by company. Information: Christina D. Cicala, President BioScreen International, Inc., 5100 Vest Lane, Waldorf, MD 20601; Tel: 301-870-7788; Fax: 202-651-8016: 202-484-4775.

Chembridge Corporation (Russia)
Chembridge offers access to a large collection of compounds (>100,000) gleaned from academic and research institutes of Russia and the former Soviet republics. Of special note is the association of Chembridge with the data management company Chemical

Design. This interesting partnership whereby Chem-X, Chemical Design's pharmacophore analysis software, is applied in the cataloging of compounds from Chembridge's extensive compound libraries and the marketing of the same in catalog formats. Compounds are provided in 96-well plate format through contract with MicroSource Discovery Systems. Information: Eugene F. Vaisberg, Chembridge Corp., 5 Revere Drive, Suite 200, Northbrook, IL 60062-9998: Tel: 708-498-7500; Fax: 708-541-2817. Chemical Design: Tel: 201-529-3323; Fax: 201-529-2443.

ComGenex, Ltd. (Hungary)
An early provider in test compound access, ComGenex maintains a library of 20,000 compounds derived primarily from synthesis. Offices have been established in the USA. Its sources have expanded from academic sources in Eastern Europe to include modular parallel synthesis techniques which they have developed in-house and market independently. ComGenex will also provide compounds in microplates through an arrangement with MicroSource Discovery Systems. Information: Ferenc Darvas, ComGenex Ltd., 5 Hollan F., Budapest Hungary 11136. Tel: 36-1-112-4878; Fax: 36-1-132-2574. US office: Tel: 609-520-0599; Fax: 609-520-0897.

Contact Service Company (Russia)
Contact Service Company draws its supplies from Russian universities and institutes. Compounds are available individually in milligram to gram quantities as well as in preselected sets in 96-well microplates. This latter offering is in association with and managed by MicroSource Discovery Systems. Information: Nikolai P. Savtchouk, POB 32, Strakhovay Uchastok, Dolgaprudny, Moscow Region 141700, Russia. Tel: 7-95-408-8051; Fax: 7-95-576-0155.

Maybridge Chemical Limited (UK) & Ryan Scientific, Inc. (USA)
Maybridge was one of the first companies to make small samples of their synthetic collection available for screening purposes. Today they offer an extensive collection (>50,000 compounds) of analogs of aryl and heterocyclic core structures, most of which are prepared in house with full synthesis back-up. Cost is based on quantity purchased. Electronic data files of their collection are available without charge. Maybridge is represented by Ryan Scientific in the US. Information: Ryan Scientific, Inc. 1323 Broad River Road, Columbia, South Carolina 29210, USA. Tel: 803-884-4911; Fax: 803-884-5568.

MicroSource Discovery Systems, Inc. (USA)
A pioneer in the provision of test compounds in 96-well microplates in 1993, MicroSource has evolved into a provider of compounds individually for its microplate customers and as a service group for the management and plating of compound collections for both brokers and discovery based companies. Their compound library is approximately 15,000 compounds in-house with a good selection of pure and novel natural products. MicroSource also provides access to ~100,000 compounds through their international network of suppliers. Screening of commercial collections is conducted prior to plating to remove analog redundancies, bio-incompatible and undesirable compound classes. Information: Mary J. Ortner, MicroSource Discovery Systems, Inc., 21 George Washington Plaza, Gaylordsville, Connecticut 06759. Tel: 860-350-8078; Fax: 860-354-5300; e-Mail: *microsourc@aol.com*

Molecular Diversity Preservation International (MDPI)
An unusual supplier that provides structure lists through their internet homepages for Europe (*http://www.unibase.ch/mdpi/database.htm*) and a parallel form for the Americas (*http://www.mdpi.org/database.htm*). The list is updated monthly. Total repository is >30,000 compounds. Information: Shu-Kun Lin, MDPI, Saengergasse 25, CH-4054 Basel, Switzerland. Tel: 41-79-322-3379; Fax: 41-61-302-8918; e-Mail: *info'a.mdpi.org*

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II. NATURAL PRODUCT EXTRACTS

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1 Combinatorial synthesis

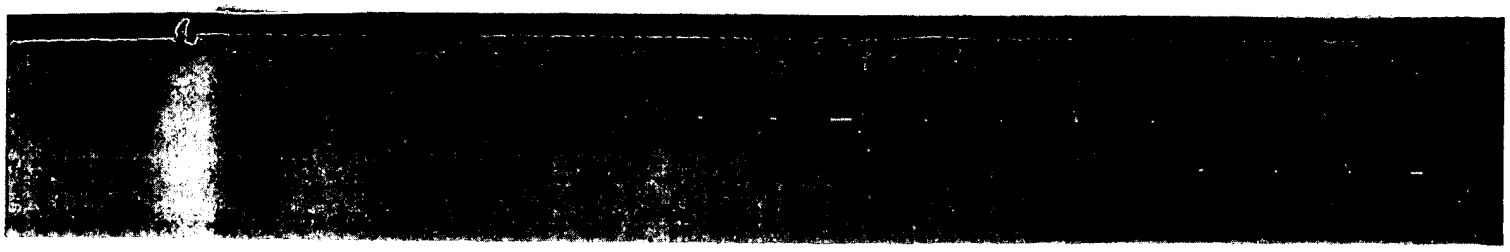
1. Introduction

Combinatorial chemistry has its earliest origins in solid phase peptide synthesis. Merrifield's Nobel Prize-winning invention depended on the use of consistent and reliable reaction conditions for peptide couplings, and the use of a polymeric solid phase to permit the simple separation of products from reagents (Merrifield 1963). The solid phase techniques have been greatly refined over the years to allow the creation of essentially any peptide molecule in a consistently high yield. In the mid 1980s, several academic teams realized that so reliable and consistent were the synthetic reactions, that the same conditions could be used to make many peptides simultaneously in the same reaction vessel. In this way, productivity could be enhanced, and the discovery of peptide epitopes responsible for protein recognition and binding could be accelerated. In particular, Houghten's use of 'tea-bags' as porous containers for solid phase resin beads, allowed the same peptide coupling step to be applied to many beads simultaneously irrespective of the sequence already attached to the bead (Houghten 1985). However, the mix and split procedure, first described by Furka (Furka *et al.* 1988), and used shortly after by Houghten (Houghten *et al.* 1991) and Lam (Lam *et al.* 1991), allowed huge numbers of peptides to be made in a very few number of chemical steps, and combinatorial chemistry was born.

In essence then, combinatorial chemistry is based on efficient, parallel synthesis, in that many more chemical compounds can be generated in a library than the number of steps used in the synthesis. This is a complete contrast to synthetic organic chemistry as it has been practised for the last 100 years, as traditional chemistry is usually distinguished by use of several synthetic steps in the preparation of one compound.

The impact of 'combichem' on both academics as well as industrial chemists has been conceptual as much as practical. The early papers in this field challenged the long-held beliefs that all synthetic chemical compounds should be made individually, in a fully purified and characterized state. Many groups have since explored high speed chemistry, both of mixtures and of single compounds, for biological applications such as drug discovery, or receptor–ligand studies, although supramolecular chemistry has also benefited. The scope of chemistry employed has branched well beyond peptide synthesis to include other key chemical transformations and structural classes. Some groups have preferred to use solution phase library chemistry (see Chapter 4) for library synthesis as this generally requires much less development and validation, but still the majority of compound libraries are made on a solid phase of some description (see Chapters 2 and 3). Resin beads are still the preferred vehicle, but grafted polymeric 'pins', paper or polymer sheets, and even glass chips have all been used.

From the outset, one major distinction can be made between mixtures and single compounds. Compound mixtures can present a highly effective method



for the discovery of compounds with particular biological or supramolecular activity, as long as there is a suitable reporting method to permit the identification of the preferred compound or compounds in the mixture. It is not possible however, to find compounds with any particular bulk property, such as magnetic or semiconducting properties, from testing mixtures. If compounds with specific bulk properties are required, it is a prerequisite to make and test isolated compounds in a pure form. Thus combinatorial chemistry methods to make discrete compounds have generally found the widest applications, whereas mixtures have mainly been applied in the search for biological activity.

With the increased productivity afforded by these new combinatorial techniques, the challenge has transferred to the efficient screening of all these products. Different groups have generated various solutions to this problem, with many elegant library designs to permit unambiguous identification of the most biologically active products. Whilst the iterative deconvolution of mix and split libraries is widely used, much invention has been applied to the use of library encoding methods. In applying this methodology, a simple chemical or analytical analysis of a tag molecule can unequivocally define the active test compound structure (Chapter 5). Encoding methods can be chemical but also they can be electronic, with the use of transponder devices that can be written and read to find the identity of preferred library components.

Thus, it can be seen that combinatorial chemistry is not one technique. It embraces a diversity of chemistry techniques: in solution or on solid phase, making libraries in mixtures or as single compounds. It may rely on esoteric encoding methodologies, or it may use none. The compounds may have been made manually, or by using the most sophisticated laboratory automation.

In addition to revolutionizing the way chemistry is now viewed and having a major impact on methods for the discovery of biologically active compounds, combinatorial chemistry has also challenged some of the science's most inventive minds to generate elegant and practical solutions to real technological problems. This book hopes to convey a sense of the diversity and invention in this area, and to illustrate how any chemist might apply some of the methods to their own work.

2. The drug discovery process

The outcome of the drug discovery process is the identification of a chemical structure that has both the desired potency against a nominated biological target, and also has suitable bioavailability and efficacy in an appropriate animal model of the targeted disease. It is this structure that the discoverer ultimately protects through a patent filing. Drug discovery is both a lengthy and expensive business for it frequently takes five years from initiating a project to the point where a potential drug is nominated for development and clinical trials.

One reason for this lengthy discovery period is that within this process, the synthesis of exploratory compounds can often be the slowest step. Early on in the lifetime of a drug discovery project medical chemists need to find a lead compound—a structure with some degree of affinity, however small, for the biological target. With this lead in hand they then proceed to the second phase: the identification of a drug development candidate by the stepwise, incremental improvement of the lead's structure (Fig. 1.1).

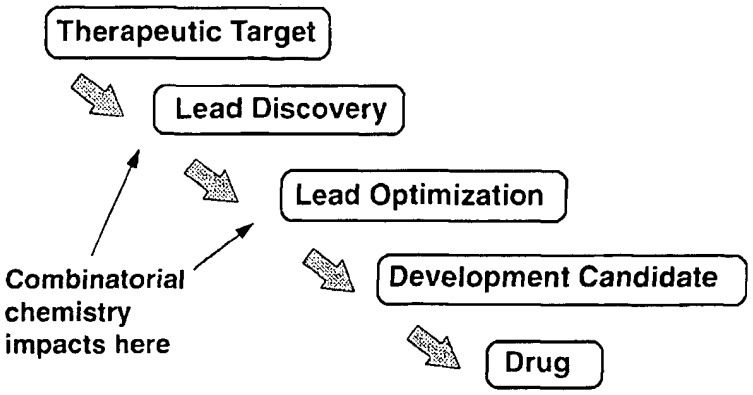


Fig. 1.1 The key steps in the drug discovery process and the steps that can be influenced by the application of combinatorial chemistry.

To address the first phase, the drug discoverer often uses a known literature or competitor's compound as a lead. But often, the target has no known ligand, and under these circumstances empirical screening may be the only approach for lead discovery. Historically, the main source of biologically active compounds for use in drug discovery programmes has been natural products, isolated from plant, animal, or fermentation sources, and the past success stories of the discovery of β-lactams, tetracyclines, avermectins, and taxol are well known. However, despite the broad range of natural product structural diversity, finding activity for a specific biological target is nearly always a highly challenging process, and companies usually resort instead to screening the compound files that they have built up over many years of drug discovery.

The average pharmaceutical company archive contains in the region of 200 000 compounds. To allow the assessment of such large numbers in a reasonable time-scale, automated methods for rapid screening have been required to allow testing of thousands of compounds per week. However, the flaw in this approach is that the structural diversity of company compound collections is limited, biased by the range of structures previously synthesized by that company. It is a moot point whether the frequent discovery of leads based on specific structural classes, benzodiazepines for example, is a consequence of their structural attributes, or whether their occurrence reflects the disproportionate number of these structures held in company vaults. The same argument could be applied to dihydropyridines (valued for their calcium channel blocking properties) or β-lactams.

We have reached a stage in the development of drug discovery where novel receptors and enzymes are increasingly being identified as therapeutic targets, but drug discoverers are restricted to screening natural product extracts or moribund compound files in order to find that elusive hint of activity that could launch a new synthetic programme. Thus any technique that could lead a new path out of this structural desert, and permit the rapid synthesis of thousands or even millions of new compounds for lead discovery would quickly capture the imagination of pharmaceutical chemists. Combinatorial chemistry provides such an opportunity, as it is a technique that in its power to

generate huge numbers of new compounds is already changing the face of drug discovery.

It has been estimated that the total number of possible small organic molecules is in the region of 10^{200} different structures (Czarnik 1995). With this total collection it might be assumed that a drug for any target may be readily discovered. But when one considers that accumulating just one molecule of each of these compounds would result in a quantity of material that exceeds the mass of the universe by a factor of 10^{128}, it can be appreciated that even empirical screening is limited by the number of compounds that realistically can be synthesized and tested.

Lead discovery from combinatorial libraries is primarily a speculative process: it has, perhaps unfairly, been called 'irrational' drug design. Initially there may be no attempt to design an active library of compounds, although library design is becoming a key factor in the synthesis of quality libraries. Rather, it is the number and variety of structures that libraries can offer that are their main attraction. Often, any structural preconceptions of what might, or should have, affinity for the target protein are usually ignored in favour of the serendipitous discovery of a novel lead. This isn't to suggest that rational design has no place in combinatorial chemistry, as any knowledge of the receptor or enzyme structure, however limited, can be used to design a library of compounds for a specific target.

Lead discovery is only one place where combinatorial chemistry is impacting on drug discovery. The second phase of taking the lead and improving its profile to the point where a drug development candidate can be nominated is also positively affected by the new high speed synthesis techniques. The chemist proceeds by formulating and testing compound binding hypotheses by synthesizing novel compounds for screening. Our understanding of the mechanisms by which compounds bind to a biological target is poorly developed, and often it is difficult to modify the lead compound in a way that reflects a rational understanding of the compound's mode of binding to the protein target. Chemists are finding that combinatorial chemistry offers a way by which many compounds can be synthesized in parallel to optimize a lead compound's activity in the absence of any binding model.

Even if the medicinal chemist has an understanding of how the lead compound works, combinatorial chemistry provides a way of rapidly exploring structure–activity relationships (SAR). For example, the chemist may recognize the need for a lipophilic side-chain on his lead molecule. Whereas in the past he may have had the time only to synthesize compounds containing methyl, propyl, and benzyl substituents, combinatorial chemistry can be used to make 100 or more analogues to map out the potential range of this substituent, allowing a more thorough investigation and possibly identifying the unexpected active analogue.

Combinatorial chemistry has changed the way we approach synthetic chemistry. In industrial environments especially where there is pressure to discover new, commercially profitable agents as quickly as possible, these techniques have already made a considerable impact. Nearly every pharmaceutical company has now established at least one group working in this area, and we have seen the emergence of huge numbers of small, specialized biotechnology companies offering new methods and applications of combinatorial synthesis.

The commercial applications of combinatorial chemistry have spawned new branches of the pharmaceutical and biotechnology industry. However, the area is not only useful commercially; it also offers an enormous intellectual challenge for industrialists and academics alike. The remainder of this chapter sets the scene for understanding this challenge by defining the basic concepts of combinatorial chemistry.

3. What is combinatorial synthesis?

The essence of combinatorial synthesis is the ability to generate large numbers of chemical compounds very quickly. This one key advantage has inspired many different uses, employing many variants of the technology. Combinatorial chemistry has also initiated a reassessment of the traditional methods of organic synthesis. Chemistry in the past has been characterized by slow, steady, and painstaking work; combinatorial chemistry has broken many of the preconceptions and permitted a level of chemical productivity undreamed of just ten years ago.

The speed of combinatorial synthesis has come about by discarding many of the dearly held precepts of organic synthesis; that all compounds and intermediates need to be fully purified and characterized. Instead by using methods that use reliable chemistry and simple but effective purifications, combinatorial chemistry has allowed great productivity although sometimes at the expense of quality. As we shall see, the pendulum is now swinging back to an equilibrium where quality becomes an equal partner to quantity, as it is no longer necessary to tolerate poor quality compounds even when made in large numbers.

How have these steps forward in productivity been taken? In the past chemists have made one compound at a time, in one reaction at a time. For example compound A would have been reacted with compound B to give product AB, which would have been isolated after reaction work-up and purification through crystallization, distillation, or chromatography (Fig. 1.2). In contrast to this approach, combinatorial chemistry offers the potential to make every combination of compound A_1 to A_n with compound B_1 to B_n. The range of combinatorial techniques is highly diverse, and these products could be made individually in a parallel fashion or in mixtures, using either solution or solid phase techniques. Whatever the technique used the common denominator is that productivity has been amplified beyond the levels that have been routine for the last hundred years.

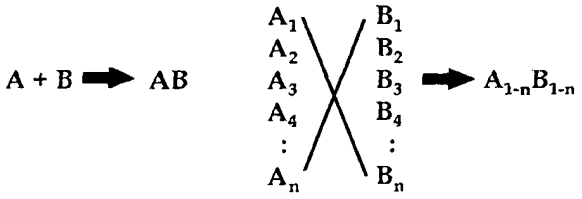


Fig. 1.2 The contrast between orthodox chemistry where products are prepared individually and combinatorial chemistry that offers the potential to prepare all the products of a range of starting materials.

In particular, the types of chemistry employed have been biased towards methods that can be expected to give good yields and purities, and furthermore there has been a reliance on techniques such as solid phase chemistry that do not require reaction work-up and also offer a degree of purification through simple physical separations such as filtration.

The next chapter describes how combinatorial chemistry evolved out of the earliest solid phase synthesis of peptides, and how simple techniques have been developed to achieve a remarkable growth in synthetic productivity.

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Bioisosterism: A Rational Approach in Drug Design

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I. Introduction

Years of cumulative research can result in the development of a clinically useful drug, providing either a cure for a particular disease or symptomatic relief from a physiological disorder. A lead compound with a desired pharmacological activity may have associated with it undesirable side effects, characteristics that limit its bioavailability, or structural features which adversely influence its metabolism and excretion from the body. Bioisosterism represents one approach used by the medicinal chemist for the rational modification of lead compounds into safer and more clinically effective agents. The concept of bioisosterism is often considered to be qualitative and intuitive.[1]

The prevalence of the use of bioisosteric replacements in drug design need not be emphasized. This topic has been reviewed in previous years.[2–5] The objective of this review is to provide an overview of bioisosteres that incorporates sufficient detail to enable the reader to understand the concepts being delineated. While a few popular examples of the successful use of bioisosteres have been included, the

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present review is focused primarily upon specific examples from current literature. The emphasis in this review was to outline bioisosteric replacements which have been used to advance drug development. No attempt was made to be exhaustive or to illustrate all of the specific analogues represented within a single study.

The ability of a group of bioisosteres to elicit similar biological activity has been attributed to common physicochemical properties. In this review an attempt has been made to quantitate, in specific instances, physicochemical effects such as electronegativity, steric size, and lipophilicity and to correlate these values to the observed biological activity. Thus, an additional objective of this review was to demonstrate the opportunities that one has in employing bioisosteres to gain more specific insight into the quantitative structure–activity relationships (QSAR) associated with a specific class of drugs. While in some instances such associations were detailed by the authors of these literature examples, others were developed on the basis of evident correlations. To further explain and rationalize the biological activity observed with nonclassical bioisosteric groups, the observed biological activity has also been correlated with some substituent constants commonly employed in QSAR studies. These observations are consistent with the fact that bioisosteric replacements often provide the foundation for the development of QSAR in drug design.[4,6] Recent advances in molecular biology, such as cloning of the various receptor subtypes, have enabled a clearer definition of the pharmacophoric sites. Bioisosteric replacements of functional groups based on this understanding of the pharmacophore and the physicochemical properties of the bioisosteres have enhanced the potential for the successful development of new clinical agents.

The bioisosteric rationale for the modification of lead compounds is traced back to the observation by Langmuir in 1919 regarding the *similarities of various physicochemical properties* of atoms, groups, radicals, and molecules.[7] Langmuir compared the physical properties of various molecules such as N_2 and CO, N_2O and CO_2, and N_3^- and NCO^- and found them to be similar. On the basis of these similarities he identified 21 groups of *isosteres*. Some of these groups are listed in Table 1. He further deduced from the octet theory that the number and arrangement of electrons in these molecules are the same. Thus, isosteres were initially defined as those compounds or groups of atoms that have the same number and arrangement of electrons. He further defined other relationships in a similar manner. Argon was viewed as an isostere of K^+ ion and methane as an isostere of NH_4^+ ion. He deduced, therefore, that K^+ ions and NH_4^+ ions must be similar because argon and methane are very similar in physical properties. The biological similarity of molecules such as CO_2 and N_2O was later coincidentally acknowledged as both compounds were capable of acting as reversible anesthetics to the slime mold *Physarum polycephalum*.[8]

Table 1. Groups of Isosteres as Identified by Langmuir

groups	isosteres
1	H^-, He, Li^+
2	O^{2-}, F^-, Ne, Na^+, Mg^{2+}, Al^{3+}
3	S^{2-}, Cl^-, Ar, K^+, Ca^{2+}
4	Cu^{2-}, Zn^{2+}
↓	↓
8	N_2, CO, CN^-
9	CH_4, NH_4^+
10	CO_2, N_2O, N_3^-, CNO^-
↓	↓
20	MnO_4^-, CrO_4^{2-}
21	SeO_4^{2-}, AsO_4^{3-}

A further extension to this concept of isosteres came about in 1925 with Grimm's Hydride Displacement Law.[9,10] This law states: "Atoms anywhere up to four places in the periodic system before an inert gas change their properties by uniting with one to four hydrogen atoms, in such a manner that the resulting combinations behave like pseudoatoms, which are similar to elements in the groups one to four places respectively, to their right." Each vertical column as illustrated in Table 2, according to Grimm, would represent a group of isosteres.

Table 2. Grimm's Hydride Displacement Law

C	N	O	F	Ne	Na
	CH	NH	OH	FH	–
		CH_2	NH_2	OH_2	FH_2^+
			CH_3	NH_3	OH_3^+
				CH_4	NH_4^+

Erlenmeyer[11] further broadened Grimm's classification and redefined isosteres as atoms, ions, and molecules in which the peripheral layers of electrons can be considered identical (Table 3).

Table 3. Isosteres Based on the Number of Peripheral Electrons

no. of peripheral electrons				
4	5	6	7	8
N^+	P	S	Cl	ClH
P^+	As	Se	Br	BrH
S^+	Sb	Te	I	IH
As^+		PH	SH	SH_2
Sb^+			PH_2	PH_3

The widespread application of the concept of isosterism to modify biological activity has given rise to the term *bioisosterism*. As initially defined by Friedman,[2] bioisosteres were to include all atoms and molecules which fit the broadest definition for isosteres and have a similar type of biological activity, which may even be antagonistic. More recently this definition has been broadened by Burger as "Compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties...".[5] The critical component for bioisosterism is that bioisosteres affect the same pharmacological target as agonists or antagonists and, thereby, have biological properties which are related to each other.

Bioisosteres have been classified as either classical or nonclassical.[12] Grimm's Hydride Displacement Law and Erlenmeyer's definition of isosteres outline a series of replacements which have been referred to as classical bioisosteres. Classical bioisosteres have been traditionally divided into several distinct categories: (A) monovalent atoms or groups; (B)

divalent atoms or groups; (C) trivalent atoms or groups; (D) tetrasubstituted atoms; and (E) ring equivalents.

Nonclassical isosteres do not obey the steric and electronic definition of classical isosteres. A second notable characteristic of nonclassical bioisosteres is that they do not have the same number of atoms as the substituent or moiety for which they are used as a replacement. Nonclassical bioisosteres can be further divided into groups: (A) rings vs noncyclic structures; and (B) exchangeable groups.

This approach to classifying bioisosteres will be used to review literature examples of those bioisosteric replacements that have provided useful information on the structure–activity relationships associated with various pharmacologically active compounds.

II. Classical Bioisosteres

A. Monovalent Atoms or Groups

Similarities in certain physicochemical properties have enabled investigators to successfully exploit several monovalent bioisosteres. These can be divided into the following groups: (1) fluorine vs hydrogen replacements; (2) amino–hydroxyl interchanges; (3) thiol–hydroxyl interchanges; (4) fluorine, hydroxyl, amino, and methyl group interchanges (Grimm's Hydride Displacement Law); (5) chloro, bromo, thiol, and hydroxyl group interchanges (Erlenmeyer's Broadened Classification of Grimm's Displacement Law).

1. Fluorine vs Hydrogen Replacements

The substitution of hydrogen by fluorine is one of the more commonly employed monovalent isosteric replacements. Steric parameters for hydrogen and fluorine are similar, their van der Waal's radii being 1.2 and 1.35 Å, respectively.[13] Thus, the difference in the electronic effects (fluorine being the most electronegative element in the periodic table) is often the basis for the major differences in the pharmacological properties of agents where fluorine has been substituted for hydrogen. Due to its electronegativity, fluorine exerts strong field and inductive effects on the adjacent carbon atom. Fluorine substitution, in general, exerts a diminished electron-withdrawing effect at distal sites. However, fluorine can donate a lone pair of electrons by resonance. This is commonly referred to as its mesomeric effect. The opposing resonance and field effects can nearly cancel. The pharmacological differences can be attributed to the influence of the electron-withdrawing effect that the fluorine substitution causes on interaction with either a biological receptor or enzyme, as well as its effect on the metabolic fate of the drug.

The antineoplastic agent 5-fluorouracil (5-FU) represents a classical example of how fluorine substitution of a normal enzyme substrate can result in a derivative which can alter select enzymatic processes. In this instance, 5-FU is biochemically transformed *in vivo* into 5-fluoro-2′-deoxyuridylic acid. Its close similarity to uracil allows this fluoro derivative to be a successful mimetic. This biochemically altered form of 5-FU, 5-fluoro-2′-deoxyuridylic acid, is ultimately responsible for the inhibition of thymidylate synthase, an enzyme involved in the conversion of uridylic acid to thymidylic acid and critical for DNA synthesis (Figure 1). The increased reactivity of 5-fluoro-2′-deoxyuridylic acid relative to 2′-deoxyuridylic acid is due to the inductive effect of fluorine which results in its covalent binding to thymidylate synthase.

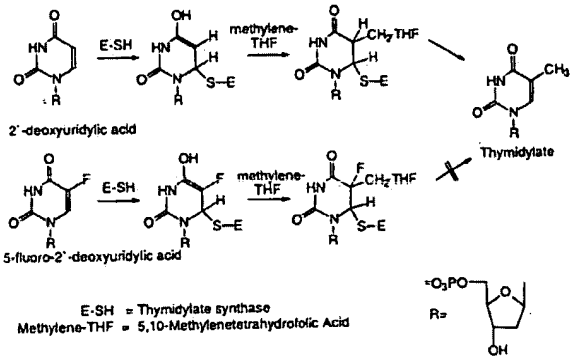
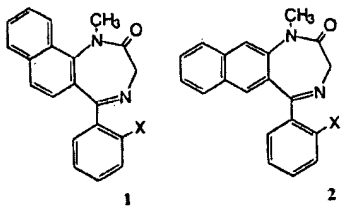


Figure 1.

The application of the monovalent substitution of a fluorine atom for a hydrogen atom can also be seen in a more recent study with naphthyl-fused diazepines, which were employed as agonistic probes of the pharmacophore of benzodiazepine receptors.[14] Replacement of the hydrogen with fluorine at the *ortho* position of the pendent phenyl group of either naphthyl-fused diazepines, as illustrated in Figure 2, resulted in enhanced affinity and efficacy for both naphthyl isomers (Table 4). This greater receptor binding affinity could again be attributed to the inductive effect of the fluorine atom facilitating a stronger interaction with the receptor.



1 2

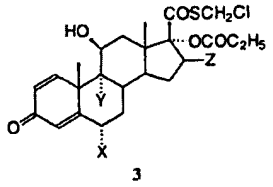
Figure 2.

Table 4. Benzodiazepine Receptor Binding Affinity for Naphthyl-Fused Diazepines

compound	X	IC_{50} (nM)[a]
1a	H	1000
1b	F	260
2a	H	1000
2b	F	55

[a] *In vitro* potency of the compound to displace [^{3}H]flunitrazepam from the benzodiazepine receptor.

Another good illustration of this monovalent bioisosteric replacement is observed in a recent series of anti-inflammatory corticosteroid analogues (**3**, Figure 3).[15] In this study, the topical anti-inflammatory activity of two pairs of structurally similar corticosteroids were compared. Their relative anti-inflammatory activity was normalized to fluocinolone acetonide, which was assigned a potency of 100. Table



3

Figure 3.

Table 5. Biological Activities of Halomethyl Androstane-17β-carbothionates

compound	X	Y	Z	topical anti-inflammatory activity[a]
3a	H	F	$=CH_2$	42
3b	F	F	$=CH_2$	108
3c	H	H	β-CH_3	27
3d	H	F	β-CH_3	41

[a] Topical anti-inflammatory activity was measured in mice by modifications of the croton oil ear assay.[16] Fluocinolone acetonide served as a positive control and is assigned a relative potency index of 100.

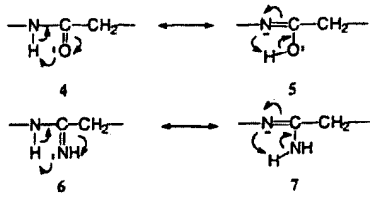
5 shows that, in the case of the pair of compounds possessing a 16-methylene substituent, the presence of an additional fluorine atom at the 6α position results in a derivative with greater activity than **3a** or fluocinolone acetonide. With the pair of corticosteroids with a 16-methyl substituent (Z = CH_3), replacement of hydrogen with fluorine at the 9α position, **3d**, also increased anti-inflammatory activity relative to **3c**.

Thus, the ability of fluorine to replace hydrogen is an effective method of exploring the affinity of an agent to the target site (receptor or enzyme) by virtue of its greater electronegativity while other parameters such as steric size and lipophilicity[17] are maintained.

2. Interchange of Hydroxyl and Amino Groups

The monovalent interchange of amino and hydroxyl groups is well known and has been successfully employed in the development of various pharmacological agents. The similar steric size (Table 7), spatial arrangement, and the ability of these functional groups to act as either *hydrogen bond acceptors or donors* is likely responsible for their successful use as bioisosteres.

Several medicinal agents under investigation as potential clinical agents carry heteroaromatic moieties. Many of these heteroaromatic compounds are capable of tautomerization. The prototropic tautomerism of heteroaromatic compounds includes all agents wherein a mobile proton can move from one site to another within the heteroaromatic molecule. Figure 4 illustrates one of the more common types of tautomerization involving the movement of a proton between a cyclic nitrogen atom and a substituent on the neighboring carbon atom within the ring. Tautomerism in heterocyclic molecules has been extensively studied.[18] In the presence of electron-donating atoms such as nitrogen in heterocyclic systems, it is known that there will be substantial tautomerization where a neighboring C—OH will tautomerize to C=O.[19] In the case of a neighboring

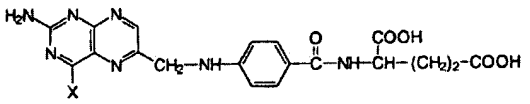


4 5 6 7

Figure 4.

carbon containing C—NH_2 (**7**, Figure 4), the preferred tautomer is the C—NH_2 form.

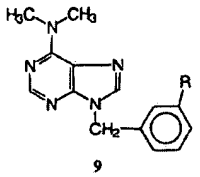
Perhaps the best known example of classical isosteric substitution of an amino for a hydroxyl group is illustrated by aminopterin (**8b**) wherein the hydroxyl substituent of folic acid (**8a**) has been substituted by an amino group (Figure 5). As previously noted, this represents a monovalent bioisosteric substitution at a carbon atom adjacent to a heterocyclic nitrogen atom. Thus, this bioisosteric replacement has the capability of mimicking even the tautomeric forms of folic acid. The similarity as well as the capability of the amino group to hydrogen bond to the enzyme are two important factors that facilitate the binding of aminopterin to the enzyme dihydrofolate reductase.



8a X = OH Folic Acid
8b X = NH_2 Aminopterin

Figure 5.

Interchange of an amino group with a hydroxyl moiety in the case of 6,9-disubstituted purines (Table 6) has been shown to result in the development of agents with similar benzodiazepine receptor binding activity.[20] This example further substantiates the ability of the amino group to mimic the hydroxyl group at the receptor site. In this study a series of 6,9-disubstituted purines were tested for their ability to bind to the benzodiazepine receptor in rat brain tissue. The relative activity of the 9-(3-aminophenyl)-methyl derivative (**9a**) was compared to the 9-(3-hydroxyphenyl)methyl analogue (**9b**) (Figure 6). In contrast to aminopterin where a dramatic difference in binding affinity was observed relative to the normal substrate, these bioisosteric 6,9-disubstituted



9

Figure 6.

Table 6. Benzodiazepine Receptor Binding Activity of Substituted 6-(Dimethylamino)-9-benzyl-9*H*-purines

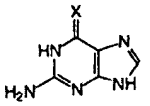
compound	R	IC_{50} (μM)[a]
9a	NH_2	0.9
9b	OH	1.2

[a] Concentration of compound that decreased specific binding of 1.5 nM [^{3}H]diazepam to rat brain receptors by 50%.

purines exhibited similar activity with regard to their affinity for the benzodiazepine receptor. In this example of bioisosteric replacement, pharmacological activity was retained. It is important to note that retention of biological activity based on *in vitro* data can be critical in those instances where differences between bioisosteric analogues exist with regard to *in vivo* parameters which may include absorption, distribution, metabolism, or elimination. While one may only observe retention of activity associated with interaction of drug with the pharmacophore, bioisosteres may differ dramatically in their *in vivo* efficacy. Additional examples of this bioisosteric replacement will be discussed in the next section on monovalent replacement of hydroxyl and thiol groups.

3. Interchange of Hydroxyl and Thiol Groups

The interchange of thiol for hydroxyl can be considered as an extension of the amino–hydroxyl replacement and has been used extensively in medicinal chemistry. This replacement is based on the ability of both these functional groups to be *hydrogen bond acceptors or donors*. A classical illustration of this replacement being guanine (**10a**) and 6-thioguanine (**10b**, Figure 7).[21]



10a X = O Guanine

10b X = S 6-Thioguanine

Figure 7.

As discussed in the previous section, when part of a heteroaromatic ring, these functional groups can exist in different tautomeric forms. Figure 8 illustrates the most common example wherein a mobile proton on a nitrogen atom in the aromatic ring can be transferred to the heteroatom attached to the adjacent carbon resulting in the different tautomers.

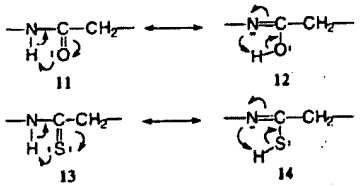


Figure 8.

In the case of 6-thioguanine, the ability of this bioisosteric analogue to be viewed as a substrate by the salvage pathway associated with purine biosynthesis, allows for its transformation into 6-thioguanylic acid by hypoxanthine-guanine phosphoribosyltransferase (HGPRT). However, the significance of this "fraudulent" nucleic acid with respect to its lethality to neoplasms is uncertain.[22] It is as this phosphoriboside that either the *de novo* synthesis of nucleic acids is inhibited or incorporation into deoxyribonucleic acid occurs.

In an attempt to enhance the calcium channel blocking capacity of certain dihydropyrimidine agents, a number of isosteric analogues with the general structure **15** (Figure 9) were synthesized.[23] Substitution of the hydroxyl with an amino resulted in

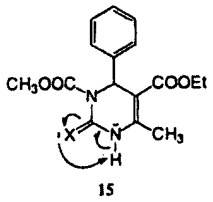


Figure 9.

Table 7. Calcium Channel Blocking Activity of 1,4-Dihydropyrimidines

compound	X	van der Waal's radius[24] (Å)	IC_{50} (nM)[a]
15a	=O	1.40	140
15b	=NH	1.50	160
15c	=S	1.85	17

[a] Concentration that produced 50% inhibition and determined for the vasorelaxant activity with potassium-depolarized rabbit thoracic aorta.

analogues with similar potency. However, substitution with the thiol resulted in enhanced potency (Table 7). This could be explained by the fact that the size of the substituents, described here as the van der Waal's radii, and the ability to hydrogen bond were the important factors influencing retention of activity. Therefore, replacement with the amino group, which has a similar size, resulted in similar potency. However, replacement with the sterically optimal thiol resulted in an analogue which was an order of magnitude more potent.

The use of this replacement in the design of novel anti-inflammatory agents substantiates its utility as a monovalent bioisostere. Long term use of nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of rheumatoid arthritis and other inflammatory diseases has been associated with side effects such as gastrointestinal ulceration, bleeding, and nephrotoxicity.[25,26] With a view to designing new drugs with an improved safety profile, certain thiazoles (**16**, Figure 10 and Table 8) that are dual

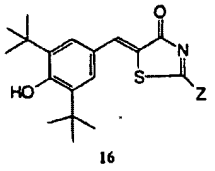


Figure 10.

Table 8. Anti-inflammatory Activity of Benzylidene Derivatives in Intact Rat Basophilic Leukemia (RBL-1) Cells

compound	Z	electronegativity[29]	IC_{50} (μM)[a] 5-LO	CO
16a	OH	3.51	1.4	0.35
16b	NH_2	2.61	0.77	0.39
16c	SH	2.32	0.38	0.012

[a] Concentration of the test compound causing 50% inhibition of 5-LO or CO formation.

inhibitors of both cyclooxygenase (CO) and 5-lipoxygenase (5-LO) are being studied as potential anti-inflammatory agents.[27] The beneficial effects of NSAIDs have been attributed to the inhibition of the enzyme cyclooxygenase, thereby preventing production of pro-inflammatory prostaglandins.[28] Leuko-

trienes produced by the 5-lipoxygenase enzyme pathway may also contribute to both inflammation and NSAID-induced effects. Table 8 summarizes the concentrations of test compounds required to cause a 50% inhibition of 5-LO and CO formation.

Replacement of the hydroxyl with an amino group resulted in more potent activity toward 5-LO while the potency toward CO remained the same. However, replacement with a thiol resulted in enhanced potency toward both 5-LO and CO. Comparison of the electronegativity values of oxygen, nitrogen and sulphur (Table 8) suggests that this could be a factor that modulates the degree of inhibition of 5-LO. Size, however, may play a significant role with regard to inhibition of CO (Table 7). Thus, the thiol group may be a suitable and informative bioisostere for the amino and hydroxyl groups in several different series of medicinal agents by the virtue of its size, lower electronegativity, and ability to hydrogen bond.

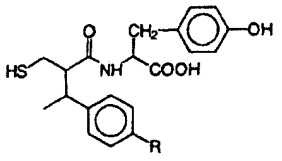
4. Fluorine and Hydroxyl, Amino, or Methyl Groups as Replacements for Hydrogen (Grimm's Hydride Displacement Law)

This monovalent group of isosteres is a result of the direct adaptation of Grimm's Hydride Displacement Law. The basis for the fluorine–hydrogen interchange and the hydroxyl–amino interchange was discussed previously. The existence of this larger group of isosteres might be attributable to a greater tolerance of the different physicochemical parameters of these functionalities within a particular series of agents. However, in the studies outlined in this section, an attempt was made to correlate a physicochemical parameter of this group of bioisosteres with the observed effect on biological activity.

In designing agents for the treatment of cardiovascular diseases, it may be beneficial to associate the hypotensive effects resulting from the inhibition of angiotensin II formation with the diuretic and natriuretic responses. Diuretic and natriuretic effects can be mediated by protection of the endogenous atrial natriuretic peptide (ANP) from inactivation by inhibition of epithelial neutral endopeptidase (NEP). Inhibition of angiotensin II formation may be brought about by inhibition of endothelial angiotensin-converting enzyme (ACE). A series of dual metallopeptidase inhibitors have been designed on the basis of the characteristics of the active sites of both enzymes. Monovalent substitution by fluorine, hydroxyl, and amino in place of hydrogen has recently been used in the design of these metallopeptidase inhibitors (Figure 11, Table 9).[30]

In this study optically pure *N*-[2-(mercaptomethyl)-3-phenylbutanoyl] amino acids (**17**) were evaluated as dual inhibitors of NEP and ACE. Substitution with isosteres (–F, –OH, $-NH_2$) as described by Grimm's Hydride Displacement Law (Table 2) resulted in retention of activity. It was observed within this series, however, that the increase in the effective van der Waal's radii of the isosteric substituents resulted in a decrease in activity (Table 9). In this instance, no significant alteration in preferential activity with either of the peptidases, ACE or NEP, was observed for these bioisosteres.

The empirical approach used to advance the structure–activity relationships with these peptidase



17

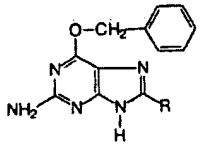
Figure 11.

Table 9. *In Vitro* Inhibition of NEP and ACE by *N*-[2-(Mercaptomethyl)-3-phenylbutanoyl] Amino Acids

			IC_{50} (nM)	
compound	R	effective van der Waal's radii (Å)[31]	angiotensin converting enzyme	neutral endopeptidase
17a	H	1.20	4.3	2.5
17b	F	1.47	6.9	5.9
17c	OH	1.53	7.9	9.0
17d	NH_2	1.79	12.0	16.0

inhibitors is useful despite the fact that a more selective ACE inhibitor was not developed. Retention of activity within this series of bioisosteres permits an assessment of the validity of a possible correlation with one or more specific physicochemical parameters. This study, for example, did provide insight into structural features which were critical to their activity as inhibitors of these peptidases.

Recently, several 8-substituted O^6-benzyl guanines (**18**, Figure 12) were evaluated for their ability to inactivate the human DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT) (Table 10).[32] Inactivation of the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase by exposure to compounds such as O^6-benzylguanine leads to a dramatic enhancement in the cytotoxic response of human tumor cells and tumor xenografts to chemotherapeutic drugs. This effect is principally observed for chemotherapeutic agents whose mechanism of action involves modification of DNA guanine residues at the O^6-position. In this study the effect of the interchange of NH_2, OH, as well as CF_3 (a bioisostere for a methyl group based on the replacement of hydrogen with fluorine) on activity was assessed. Analogues possessing electronegative groups at the 8-position were more effective as inactivators of AGT in human HT29 colon tumor cell extracts. The relative activities of these bioisosteres based on the dose required for 50% inhibition (ED_{50}) along with their electronegativities are outlined in Table 10.



18

Figure 12.

Table 10. Alkyl Guanine Transferase Inactivating Activity of 6-(Benzyloxy)purine Derivatives

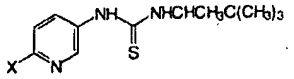
compound	R	electronegativity[29]	ED_{50} (μM)[a]
18a	NH_2	2.61	2.0
18b	CF_3	3.46	0.25
18c	OH	3.51	0.15

[a] Effective dose required to produce 50% inactivation in HT-29 cells upon incubation for 4 h.

Again, the retention of activity within a series of bioisosteres provides the basis for the discovery of a possible correlation between pharmacological activity and the physicochemical properties of specific agents.

As an extension to the above group defined by Grimm's Hydride Displacement Law, the widespread use of the chlorine atom as a bioisostere has been observed in several different series of biologically active compounds. This could be attributed to the similarity in size between these atoms, a comparison of which is made in Table 11. Further, there exists similarity in the lipophilicity of the methyl group with that of chlorine which may be responsible for its suitability as a monovalent bioisosteric replacement.

N-(Substituted-3-pyridyl)-*N'*-alkylthioureas (**19**, Figure 13), which have been evaluated as novel potassium channel openers,[33] are among the more recent illustrations of the replacement of chlorine with isosteres from Grimm's Hydride Displacement Law (Table 11). Potassium channel openers cause vasorelaxation in vascular smooth muscle through hyperpolarization of the cell membrane. There is an increased interest in these compounds based on their therapeutic potential in the treatment of cardiovascular diseases. Substitution at the 6-position with monovalent isosteres ($-NH_2$, $-CH_3$, $-Cl$) results in analogues with similar biological activity. It was observed that substituents with similar biological activity had comparable effective van der Waal's radii (Table 11). The methyl group, which has a lower electronegativity, elicited a weaker pharmacological response, suggesting an additional correlation between activity and a physicochemical property.



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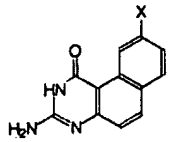
Figure 13.

Table 11. Inhibition of Spontaneous Mechanical Activity in Rat Portal Vein (*in vitro*)

compound	X	maximum fall in SBP[a] (%)	electronegativity[29]	effective van der Waal's radii[31] (Å)
19a	NH_2	29	2.61	1.79
19b	CH_3	18	2.27	1.80
19c	Cl	27	3.0	1.73

[a] Antihypertensive activity measured as maximum % fall in systolic blood pressure in anesthetized normotensive rat by iv injection.

Table 12 lists the relative potency of a group of bioisosteres which act as inhibitors of thymidylate synthase. Each of these benzo[*f*]quinazolin-1(2*H*)-ones (**20**, Figure 14), inhibit thymidylate synthase by virtue of their structural relation to its cofactor, 5,10-methylenetetrahydrofolic acid.[34] They are, therefore, referred to as folate-based thymidylate synthase inhibitors. These analogues differ from other folate-based thymidylate synthase inhibitors as the absence of a glutamate residue suggests that they are not dependent upon active folate transport and polyglutamylation for activity, the two mechanisms of resistance that have been observed with agents such as methotrexate. Within this series, it was observed that hydrogen bond donors were more potent than the unsubstituted parent compound. These analogues, however, were less active than compact lipophilic groups in elevating thymidylate synthase inhibition. Thus, at the 9-position, optimal size and lipophilicity appear to be critical factors associated with their ability to inhibit thymidylate synthase.



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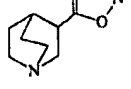
Figure 14.

Table 12. Thymidylate Synthase Enzyme Inhibition Data for Benzo[f]quinazolin-1(2*H*)-ones

compound	X	thymidylate synthase inhibitory activity IC_{50} (μM)[a]
20a	Cl	0.025
20b	CH_3	0.178
20c	OH	0.48
20d	NH_2	0.63
20e	H	1.08

[a] Inhibitor concentration producing 50% inhibition and determined by the tritium release assay of Roberts[35] as modified by Dev et al.[36]

In another study aimed at designing cholinergic agents which would be capable of penetrating the central nervous system and displaying high efficacy at the cortical muscarinic receptors, a series of oxadiazole-based tertiary amines **21** (Figure 15, Table 13) were tested. The assay used was designed to measure affinity and predict cortical efficacy from the antagonist−agonist (i.e. NMS/OXO-M) binding ratio in rat cortical membranes.[37] The log of this ratio has been shown to correlate with the ability of the ligand to stimulate the hydrolysis of cortical phosphatidylinositol. It is known that at least three muscarinic receptor subtypes m1, m3, and m5 may be capable of positively stimulating phosphatidylinositol hydrolysis. In this series it was observed that replace-



21

Figure 15.

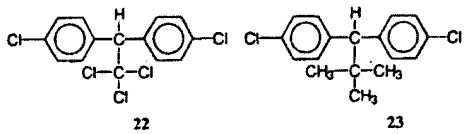
Table 13. *In Vitro* Binding Data for Substituted Oxadiazoles

compound	X	K_a(app) (μM) [^{3}H]NMS[a]	[^{3}H]OXO-M[b]	ratio[c]
21a	CH_3	0.44	0.0009	490
21b	Cl	0.19	0.00048	400
21c	NH_2	0.60	0.0005	1200

[a] Displacement of [^{3}H]-*N*-methylscopolamine. [b] Displacement of [^{3}H]oxotremorine-M. [c] Ratio of K_a(app) for NMS to K_a(app) for OXO-M.

ment of the methyl with the less lipophilic amino group resulted in an increase in this antagonist–agonist binding ratio. However, replacement with a chloro substituent which is isosteric and isolipophilic to the methyl group resulted in a compound which had lower binding affinity, but a very similar antagonist–agonist binding compared to the methyl-substituted analogue.

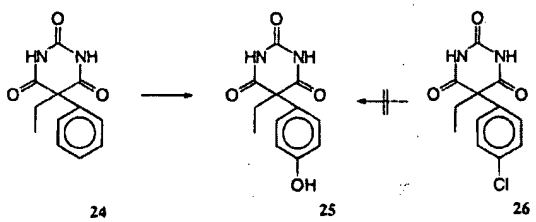
While the chlorine atom is often viewed to be isosteric and isolipophilic with the methyl group, it is very often selected as a bioisosteric replacement because of its ability to alter the metabolism. Previous research has very effectively illustrated the feasibility of altering the metabolism of an agent by the interchange of chlorine with one of the bioisosteres from Grimm's Hydride Displacement Law. Replacement of a chloro atom with a methyl substituent can facilitate metabolism of a xenobiotic. Lipid-soluble chemicals tend to be distributed into adipose tissue where, unless they are metabolized, they tend to accumulate for long periods of time, e.g. DDT (**22**, Figure 16). The replacement of the trichloromethyl moiety with a *tert*-butyl group (**23**, Figure 16) results in diminished persistence of this pesticide.[38] The methyl substituents provide a site which is susceptible to metabolic degradation.



22 23

Figure 16.

Alternatively, the addition of a chloro substituent can be effective in inhibiting metabolic oxidation. Phenobarbital (**24**) is metabolized by aromatic hydroxylation at the *para* position to produce **25** (Figure 17), which then forms a glucuronide conjugate. Replacement of the hydrogen at the *para* position with chlorine (**26**) prevents this route of metabolism. Thus, in this instance the chlorine atom blocks the metabolism of phenobarbital and thereby increases its duration of action.



24 25 26

Figure 17.

2-(Diethylamino)ethyl benzoates (**27**, Figure 18), which are used as local anesthetics provide yet another example where classical bioisosteric replacement affects the observed duration of action by altering metabolism.[39] On the basis of the results outlined in Table 14, it appears that the decreased half-lives ($t_{1/2}$) associated with those analogues having electron-withdrawing groups is likely associated with their increased susceptibility to hydrolysis.



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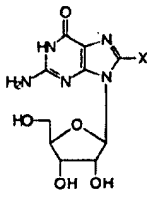
Figure 18.

Table 14. Biological Half-Lives of *Para*-Substituted 2-(Diethylamino)ethyl Benzoates

compound	X	σ_p	$t_{1/2}$
27a	NH_2	−0.66	96.29
27b	OCH_3	−0.26	26.75
27c	Cl	0.23	4.94
28d	CN	0.63	1.28

5. Monovalent Substitutions Involving Chloro, Bromo, Thiol, and Hydroxyl Groups (Erlenmeyer's Broadened Classification of Grimm's Displacement Law)

The last class of monovalent isosteres involves chloro, bromo, thiol, and hydroxyl group interchanges. While we have already rationalized the hydroxyl–thiol interchanges, the classification of the chloro, bromo and thiol group together is based on their similarity in their number of peripheral electrons as defined by Erlenmeyer. Use of these monovalent isosteric replacements is illustrated for certain C8-substituted guanosine analogues (**28**, Figure 19). To evaluate the efficacy of C8-substituted guanosine analogues in the enhancement of primary antibody response *in vitro*, these analogues were cultured with antigen and adjuvant.[40] B lymphocytes were incubated with 1.0 mM of the guanosine analogues in presence or absence of type 2 antigen TNP-Ficoll and after 3 days the cells were processed, collected, and then evaluated for the number of antibody-forming cells. The results of this study demonstrated that the bromo-, hydroxyl-, and thio-substituted analogues could stimulate polyclonal immunoglobulin secretion and in the presence of antigen enhanced the magnitude of the anti-TNP plaque-forming cell (Anti-TNP PFC) antibody response to antigen TNP-Ficoll as summarized in Table 15. The thiol bioisostere was more potent as compared to the hydroxyl and the bromo isosteres.



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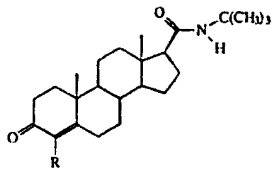
Figure 19.

Table 15. Enhancement of Antibody Responses of DBA/2 B Cells by Guanosine Analogs

		antigens: no. of anti TNP plaque-forming cells/culture	
compound	X	medium	TNP-Ficoll
28a	medium	14	8
28b	medium + Br analogue	78	198
28c	medium + OH analogue	118	200
28d	medium + SH analogue	80	296

Dihydrotestosterone is a potent androgen known to be essential for male differentiation, growth, and function of androgen-sensitive sex organs and is involved in the pathophysiology of many diseases

such as benign prostatic hyperplasia and prostrate cancer. Inhibition of steroid 5α-reductase is of recent pharmaceutical interest in view of its role in the conversion of testosterone to dihydrotestosterone. Recent research has isolated and characterized two types of human 5α-reductases, namely type I and type II. Therefore, a rational approach for treatment of these androgen-sensitive disease states can be envisioned by the inhibition of either one or both enzymes with specific inhibitors. 4-Substituted *N*-(1,1-dimethylethyl)-3-oxo-4-androsten-17β-carboxamide analogues (**29**, Figure 20) with hydroxy, thiol, chlorine, or bromo substituents at the 4-position were evaluated and found to show intermediate inhibitory activity on human type II 5α-reductase activity.[41] From the data obtained (Table 16), it could be inferred that the enzyme has steric and electronic preferences at this position, resulting in significantly enhanced potency for the hydroxy and chloro substituted compounds.



29

Figure 20.

Table 16. *In Vitro* Inhibition of Human Type II 5α-Reductase of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17β-carboxamides

compound	R	effective van der Waal's radii[31] (Å)	electro-negativity[29]	IC_{50} (nM)
29a	OH	1.53	3.51	172
29b	Cl	1.73	3.00	192
29c	SH	1.80	2.32	437
29d	Br	1.86	2.80	387

[a] IC_{50} values represent the concentration of compounds required to inhibit 5α-reductase activity by 50%.

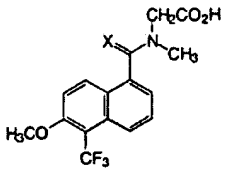
B. Divalent Isosteres

Divalent isosteres can be classified into two subgroups: (1) Those divalent bioisosteres which involve the interchange of atoms that are involved in a double bond, such as in the series; C=C, C=N, C=O, and C=S. And (2) those divalent isosteres where substitution of a different atom results in the alteration of two single bonds such as in the series; C−C−C, C−NH−C, C−O−C, and C−S−C. Both of these types of bioisosteric substitutions have been used extensively in the study of the structure−activity relationships of various pharmacologically active agents.

1. Divalent Replacements Involving Double Bonds

This subclass includes replacements such as C=S, C=O, C=NH, and C=C. As we have already discussed in the earlier sections, the presence of heterocyclic systems in most of the lead compounds under study as medicinal agents facilitates the tautomerization of these groups. As mentioned earlier, for the purpose of this review, the classification of such analogues as divalent bioisosteres requires the absence of a mobile proton which can migrate within the ring system.

The replacement of C=S with C=O in Tolrestat (**30a**, Figure 21), an aldose reductase inhibitor currently under study in human subjects for the treatment of diabetic neuropathy, resulted in oxo-Tolrestat (**30b**), which retained activity both *in vitro* and *in vivo* (Table 17).[42]



30

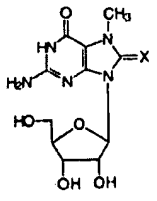
Figure 21.

Table 17. Aldose Reductase Inhibitory Activity of Tolrestat and Oxo-Tolrestat

		aldose reductase inhibition	
compound	X	*in vitro*[a]	*in vivo*[b]
30a	S	94	53
30b	O	86	56

[a] Inhibition of enzyme activity in partially purified bovine lens preparation. [b] Inhibition of galactitol accumulation in the sciatic nerves of rats fed 20% galactose for 4 days.

Another illustration of the use of this class of divalent isosteres is with certain novel purine nucleoside analogues (**31**, Figure 22) that were tested *in vivo* for antiviral activity against Semliki Forest virus (SFV) infection in a mouse model.[43] Table 18 outlines the percentage of mice that survived for 21 days for the various bioisosteric analogues compared to the absence of any survivors in control infected mice. Replacement of the sulfur atom at C-8 with the oxygen or selenium atom, another well-known divalent isosteric replacement, resulted in weaker activity relative to the thio analogue.



31

Figure 22.

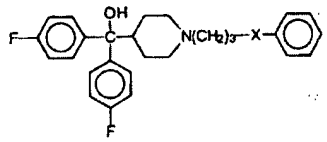
Table 18. Activity of Guanosine Bioisosteric Analogues against Semliki Forest Virus

compound	X	% total survivors
31a	S	83 (10/12)
31b	O	67 (8/12)
31c	Se	58 (7/12)

2. Divalent Replacements Involving Two Single Bonds

The second major class of divalent bioisosteres represents those atoms or groups which are attached to different substituents. As these divalent bioisosteres are attached to two different substituents, the chemical and polar differences are less pronounced.

The bond angle or the conformation associated with the use of these divalent bioisosteres may be an important factor associated with retention of biological activity. Table 19 shows a comparison of the antiallergy activity and bond angles of several divalent bioisosteric substitutions which have been investigated for a series of 4-(diarylhydroxymethyl)-1-[3-(aryloxy)propyl]piperidines (**32**, Figure 23).[44]



32

Figure 23.

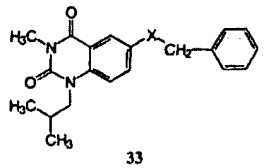
Table 19. Oral Antiallergy Activity in the Passive Foot Anaphylaxis Assay of Analogues Containing Varied Heteroatoms

compound	X	electro-negativity[29]	bond angle[45] (deg)	passive foot anaphylaxis assay (10 mg/Kg)[a]
32a	−O−	3.51	108.0	+++
32b	−S−	2.32	112.0	+
32c	$-CH_2-$	2.27	111.5	+
32d	−NH−	2.61	111.0	+

[a] Aminophylline orally at 100 mg/kg was used as a positive control and assigned a biological response of (++); (−) not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test; (+) activity between positive and negative groups; (++) activity equivalent to positive control group; (+++) activity greater than positive control group.

These compounds were tested using the passive foot anaphylaxis assay which is an IgE-mediated model useful in the detection of compounds possessing antiallergic activity. A significant correlation between biological activity and electronegativity was observed for these analogues.

Another illustration of divalent bioisosteric linkers is observed in the study of inhibitors of the nuclear factor of activated T cells (NFAT)-mediated transcription of β-galactosidase.[46] T cells are essential components of the immune response. They are activated upon contact with foreign substances, or antigens, present on invading organisms. One of the earlier events that occurs after T cells recognize a foreign antigen is the induction of the interleukin-2 (IL-2) gene. IL-2 is an essential autocrine growth factor for T cells and its appearance marks the commitment of the T cell toward activation. These activated cells release a variety of bioactive molecules which initiate a cascade of events which initiate an immune/inflammatory response. The region 257−286 base pairs upstream of the IL-2 structural gene binds to a protein, the nuclear factor of activated T cells-1 (NFAT-1), prior to IL-2 gene transcription. NFAT-1 is expressed in relatively few cells besides T cells and is markedly upregulated upon stimulation of the T cell receptor. This makes it a highly specific target within activated T cells. When the cell is activated, the NFAT-1 protein binds to the DNA at its recognition site and induces the transcription of β-galactosidase. This study evaluated some of the bioisosteric analogues of quinazolinediones (**33**, Figure 24) as potential immunosuppressive agents by their ability to inhibit β-galactosidase expression as summarized in Table 20. Here again the similar bond angles and electronegativities (Tables 19 and 20) of the −NH− and $-CH_2-$ bioisosteric linkers result in analogues which retain activity. In this study, the use of an oxygen atom as a bioisosteric linker, which has a marginally smaller bond angle and much greater electronegativity, results in an analogue with increased potency.



33

Figure 24.

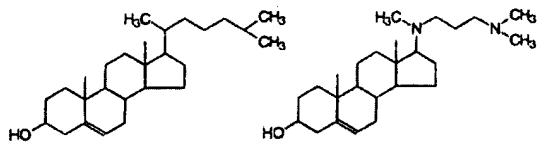
Table 20. Regulation of NFAT-1-Regulated β-Galactosidase Activity by Quinazolinediones

compound	X	IC_{50} (μM)
33a	−NH−	4.47
33b	$-CH_2-$	4.03
33c	−O−	2.5

Other divalent linkers that have been obtained as modifications of the above classical isosteres include higher oxidation states of the thioether linker resulting in sulfoxide and sulfone derivatives. These types of replacements will be discussed in the section on nonclassical isosteres.

C. Trivalent Atoms or Groups

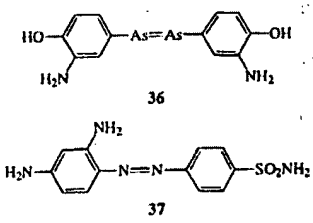
A classical trivalent bioisosteric replacement is −CH= with −N=. This replacement has been widely used in the drug discovery process and has been further discussed among the ring equivalent class of classical bioisosteres. This replacement when applied to cholesterol (**34**) resulted in 20,25-diazacholesterol (**35**, Figure 25) which is a potent inhibitor of cholesterol biosynthesis.[47] The greater electronegativity of the nitrogen atom could be responsible for the biological activity of this bioisostere.



34 **35**

Figure 25.

Another trivalent substitution based on Erlenmeyer's definition of the similarity in the number of peripheral electrons would be the replacement of −P= (bond angle C−P−C = 100 ± 4°) with −As= (bond angle C−As−C = 96 ± 5°).[48] Arsenic is a classical bioisostere of nitrogen (Table 3). Arsenicals have received considerable attention due to their therapeutic significance. The oxidation of arseno compounds to arsenoxides is important in the bioactivation of a number of chemotherapeutic arsenicals. One of the first drugs used clinically was arsphenamine (**36**, Figure 26). The activity of arsphenamine against the syphilis organism was attributed to its oxidized metabolite oxophenarsine. However,



36

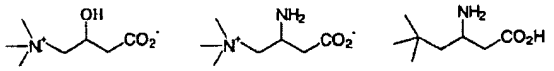
37

Figure 26.

the lack of selective toxicity associated with these arsenicals has led to a reduced interest in such replacements in current research. Analogy is drawn to prontosil (**37**, Figure 26) which is found to be metabolized to *p*-aminobenzenesulfonamide. Prontosil is inactive against microorganisms *in vitro* but active *in vivo*.

D. Tetrasubstituted Atoms

One of the more widely used tetravalent replacements has been the interchange of a quaternary charged nitrogen atom with a tertiary carbon atom. C_2–C_{20} acyl groups of acyl-CoAs are reversibly transferred to the β-hydroxyl group of (*R*)-carnitine by carnitine acyltransferases. The selective inhibition of individual carnitine acyltransferases may be useful in the therapy of diabetes and heart disease. The determination of relative substrate specificity and the development of specific inhibitors for individual carnitine acyltransferases has been of considerable interest because of its possible therapeutic implications. Certain simple acylcarnitine analogues are potent carnitine acyltransferase (CAT) inhibitors.[49] Structure–activity studies in this series have included the bioisosteric replacement of the hydroxyl group of carnitine (**38**) with an amino (**39**) and replacement of the tetravalent trimethylammonium group with a tertiary butyl group (**40**, Figure 27). Table 21 lists the similar kinetic constants that were obtained for these bioisosteres.



38 39 40

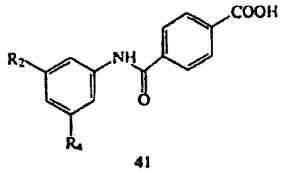
Figure 27.

Table 21. Rate Constants for Carnitine and Synthetic Analogues with Pigeon Breast Carnitine Acyltransferase

X	K_i (mM)[a]
39	4.0
40	2.6

[a] K_i values were obtained from a plot of apparent K_m vs [inhibitor].

Other tetravalent bioisosteric replacements have been investigated which use members of the same group of the periodic table (group IVB), i.e. the silicon or germanium atom. These atoms are also tetravalent and have similar hydrophobicity, but possess different electronic and steric properties from carbon. The ability of trimethylsilyl- or trimethylgermyl-containing retinobenzoic acids (**41**, Figure 28) to induce differentiation of the human promyelocytic leukemia cell line HL-60 to mature granulocytes was examined as a measure of retinoidal activity.[50] Compounds with a trimethylsilyl or trimethylgermyl group at the *meta* positions showed similar activities as the corresponding retinobenzoic acids with a *tert*-butyl group. Results in Table 22 summarize the differentiation-inducing activity observed for one such series of retinobenzoic acids.



41

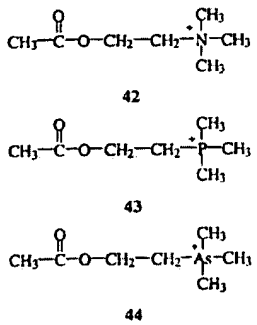
Figure 28.

Table 22. Differentiation-Inducing Activity of Trimethylsilyl- or Trimethylgermyl-Containing Retinoids

compound	R_2	R_4	ED_{50}[a]
41a	*tert*-butyl	*tert*-butyl	3.6×10^{-8}
41b	trimethylsilyl	trimethylsilyl	3.0×10^{-8}
41c	trimethylgermyl	trimethylgermyl	4.2×10^{-8}

[a] Effective dose required to cause differentiation-inducing activity in 50% of human promyelocytic leukemia cells.

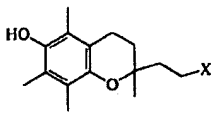
A classical illustration of tetrasubstituted isosteres involves replacement of the quaternary ammonium group in case of cholinergic agonists (**42**, Figure 29) with the phosphonium and arsonium analogues.[51] In this study, it was observed that such replacements resulted in less potent analogues with greater toxicity. Activity was found to decrease as size of the onium ion increased. The decreased potency and greater toxicity of these higher elements has diminished interest in replacements of this type for the development of direct-acting cholinergic agonists.



42 43 44

Figure 29.

A more recent illustration of retention of activity within a series of charged isosteres, as described by Erlenmeyer (Table 3) was observed for a series of α-tocopherol analogues (**45**, Figure 30) that were found to scavenge lipoperoxyl and superoxide radicals *in vitro* and accumulate in heart tissue as demonstrated by measurement of *ex vivo* inhibition of lipid peroxidation in mouse heart homogenates.[52] Table 23 illustrates that all the bioisosteric analogues of α-tocopherol were found to elicit similar biological activity.



45

Figure 30.

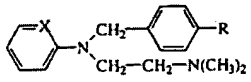
Table 23. *In Vitro* and *ex Vivo* Inhibition of Lipid Autooxidation in Mouse Heart Homogenate

compound	X	*in vitro*[a] IC_{50} (μM)	*ex vivo*[b] ID_{50} (μM/kg)	IC_{50}/ID_{50}
45a	$-N^+(CH_3)_3$	19	11	1.7
45b	$-P^+(CH_3)_3$	10	8	1.3
45c	$-S^+(CH_3)_2$	7	6	1.3

[a] Concentration that inhibits thiobarbituric acid reactive substances (TBARS) formation by 50%. [b] Dose that inhibits TBARS formation by 50% 1 h after sc administration.

E. Ring Equivalents

Ring equivalent bioisosteres represent the final subclass of classical bioisosteres that will be reviewed. Classical isosteric substitutions when applied within ring systems result in different heterocyclic analogues which can be effective bioisosteres. The use of the classical bioisosteres benzene, thiophene, and pyridine resulted in analogues with retention of biological activity within different series of pharmacological agents. One of the successful uses of this replacement resulted in the potent antihistamine mepyramine (**47**, Figure 31) which evolved by the replacement of the phenyl moiety in antegran (**46**) by a pyridyl group.

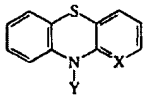


46 X = CH, R = H Antergan

47 X = N, R = OCH_3 Mepyramine

Figure 31.

Substitution of benzene by pyridine also improved activity in the tricyclic antihistamines [promethazine (**48a**) and isothipendyl (**48b**)] and neuroleptics [promazine (**48c**) and prothipendyl (**48d**)] (Figure 32) and resulted in a reduction of both sedative and extrapyramidal effects. In reviewing some more recent studies, this subclass of bioisosteres will be divided into (1) divalent ring equivalents and (2) trivalent ring equivalents.



48a Y = $CH_2CH(CH_3)N(CH_3)_2$ X = CH

48b Y = $CH_2CH(CH_3)N(CH_3)_2$ X = N

48c Y = $CH_2CH_2CH_2N(CH_3)_2$ X = CH

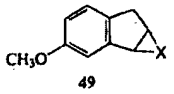
48d Y = $CH_2CH_2CH_2N(CH_3)_2$ X = N

Figure 32.

1. Divalent Ring Equivalents

Some of these heterocyclic rings obtained by the replacement of a heteroatom within different ring systems will be discussed among the nonclassical category of bioisosteres on the basis of their ability to mimic the spatial conformation of certain other functional groups.

A copper-containing monooxygenase, dopamine β-hydroxylase, present in a variety of mammalian tissues, catalyses the benzylic hydroxylation of dopamine. Since dopamine β-hydroxylase plays an important role in the biosynthetic production of noradrenaline, it could be a target for the design of inhibitors as potential therapeutic agents for the modulation of adrenergic activity *in vivo*. Isosteric divalent ring replacements as obtained from Grimm's Hydride Displacement Law (Table 2) resulted in retention of activity within a series of indane derivatives (**49**, Figure 33) evaluated as inhibitors of dopamine β-hydroxylase[53] (Table 24).



49

Figure 33.

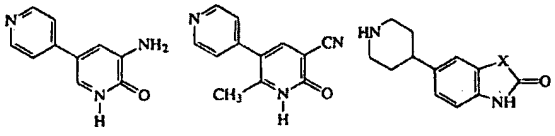
Table 24. Kinetic Inhibition Parameters for Dopamine β-Hydroxylase by Indan Derivatives

compound	X	K_i (mM)[a]
49a	$-CH_2-$	1.12
49b	$-O-$	0.65
49c	$-NH-$	0.4

[a] Apparent binding constant K_i estimated by plotting $1/k_{obs}$ as a function of 1/[I].

The applicability of divalent ring-equivalent isosteres has also been demonstrated in other five- and seven-membered ring systems. The search for novel cardiotonic agents resulted in the successful development of two clinically useful agents, amrinone[54] (**50**) and milrinone[55] (**51**) (Figure 34). It is known that the positive vasodilatory actions of amrinone and milrinone are related to the inhibition of adenosine 3',5'-cyclic phosphate phosphodiesterase III (cAMP PDE III).

During SAR studies on amrinone (**50**), it was observed that a free amino group was not necessary for *in vitro* cAMP PDE III activity.[56] This prompted the design and synthesis of analogues with general structure **52**.[57] Table 25 summarizes the *in vitro* cAMP PDE III activity of the divalent ring-equivalent



50 **51** **52**

Figure 34.

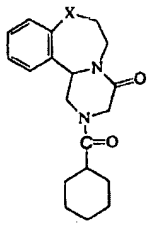
Table 25. *In Vitro* cAMP PDE III Activity of Ring-Equivalent Bioisosteres of 5-(4-Pyridinyl)benzoxazol-2(3*H*)-one

compound	X	cAMP PDE III IC_{50} (μM)[a]
50	amrinone	28
51	milrinone	0.36
52a	$-O-$	9.8
52b	$-CH_2-$	5.3
52c	$-NH-$	1.3
52d	$-S-$	0.54

[a] Concentration required to cause 50% inhibition of cAMP PDE III *in vitro*.

isosteres that were evaluated in this study. No clear correlation with physicochemical parameters could be extrapolated from this series. However, cAMP PDE inhibitory activity was retained by all the bioisosteric analogues of this series.

Divalent isosteric ring substitutions of the pyrazino-[2,1-*a*][2]benzazepine system (**53**, Figure 35) resulted in derivatives containing different heterocyclic systems. All these bioisosteres exhibited anthelmintic activity.[58] Table 26 shows a relative comparison of the MIC values of the various divalent isosteres. Replacement of the methylene with a sulfur or oxygen atom resulted in analogues with decreased potency relative to the carbocyclic analogue.



53

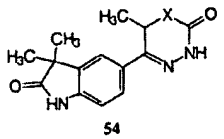
Figure 35.

Table 26. Anthelmintic Potency of Derivatives of the Pyrazino[2,1-*a*][2]benzazepine System

compound	X	minimum inhibitory concentration,[a] *Taenia crassiceps*
53a	$-CH_2-$	+++
53b	−O−	++
53c	−S−	+

[a] Concentration required to prevent all movement of cysts and evaginated scoleces: ++++ = MIC < 0.1 μg/mL; +++ = 0.1 μg/mL < MIC < 1.0 μg/mL; ++ = 1.0 μg/mL < MIC < 10 μg/mL; + = MIC > 10 μg/mL.

Replacements using selenium have been less commonly used as a divalent ring equivalent. However ring substitution of −Se− has frequently resulted in retention of their respective biological activities. Replacement with selenium has been shown to introduce a potential risk of toxicity. Substitution with the different divalent isosteres including selenium in a novel class of cardiotonic agents **54** (Figure 36 and Table 27) resulted in retention of activity.[59]



54

Figure 36.

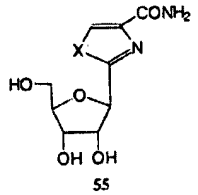
Table 27. Biochemical Properties of Heterocyclic Indolones

compound	X	IC_{50}[a] (μM)
54a	−NH−	0.17
54b	$-CH_2-$	0.24
54c	−S−	0.33
54d	−Se−	0.54
54e	−O−	0.96

[a] Concentration required to produce 50% inhibition of the sarcoplasmic reticulum bound low-K_m cAMP phosphodiesterase (SR-PDE).

The inhibition of cardiac phosphodiesterase, which is related to the development of cardiotonic agents, was assessed on the canine cardiac sarcoplasmic reticulum-bound, cGMP-inhibited, high-affinity cAMP-PDE (SR-PDE). Table 27 shows a comparison of the inhibition of phosphodiesterase (PDE) by the various divalent bioisosteric ring substitutions.

C-Glycosyl nucleosides which are analogues of nicotinamide nucleoside are expected to be converted to the analogues of NAD coenzyme and to inhibit NAD-dependent inosine monophosphate dehydrogenase (IMPD). Inhibition of this enzyme produces the accumulation of IMP and the depletion of guanine nucleotides, which is linked to DNA synthesis inhibition. Both 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin, **55b**) and 2-β-D-ribofuranosylselenazole-4-carboxamide (selenazofurin, **55c**) (Figure 37) are metabolized to analogues of NAD and have pronounced antitumor activity in animals and broad spectrum antiviral activity.[60] Selenazofurin is about 10 times more active than tiazofurin with a similar spectrum of antitumor activity (Table 28).



55

Figure 37.

Table 28. Bioisosteres of 2-β-D-Ribofuranosyl-4-carboxamides

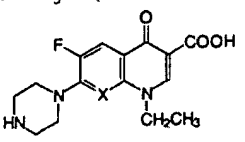
compound	X	antitumor agent	relative activity, IC_{50}[a] (μM)
55a	−O−	oxazofurin	inactive
55b	−S−	tiazofurin	1.0
55c	−Se−	selenazofurin	0.1

[a] Estimated relative potency against P388 and L1210 leukemias. Inactive until a maximum tested concentration of 4×10^{-3} M.

Replacement with oxygen in 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin) resulted in 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **55a**). Oxazofurin lost the ability to inhibit the growth of P388 and L1210 murine leukemia and HL 60 human promylelocytic leukemia.[61] This may be attributed to the lower basicity of the oxazole moiety compared to that of the thiazole moiety of tiazofurin.[62]

2. *Trivalent Ring Equivalents*

The trivalent substitution of −CH= with −N= is commonly used in modern drug design. Trivalent ring substitution of −CH= with −N= in the antibacterial agent norfloxacin (**56a**) resulted in enoxacin (**56b**, Figure 38) which is also in clinical use for its antibacterial activity[63] (Table 29).



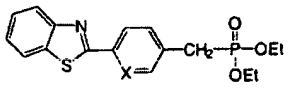
56

Figure 38.

Table 29. Isosteres of Clinically Significant Quinolone Antibacterials

compound	X	antibacterial agent
56a	−CH−	norfloxacin
56b	−N−	enoxacin

Another example of such a replacement is illustrated for the (benzothiazolylbenzyl)phosphonate derivatives (**57**, Figure 39) which retained vasodilatory activity.[64] Table 30 outlines the percent increase in coronary flow obtained by replacement of the benzene ring with pyridine.



57

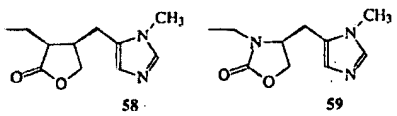
Figure 39.

Table 30. Effect of Phosphonates on Coronary Flow of Isolated Guinea Pig Heart

compound	X	maximum increase in coronary flow[a] (%)
57a	−CH−	79.9
57b	−N−	94.0

[a] Langendorff's method in isolated guinea pig heart.

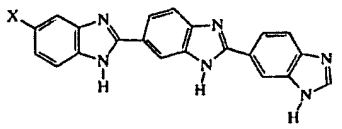
Pilocarpine (**58**, Figure 40) is widely employed as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma. The duration of lowering of intraocular pressure by pilocarpine lasts only for about 3 h. This short duration of action is mainly due to the hydrolytic cleavage of the lactone ring, resulting in the formation of pilocarpic acid and rapid elimination and/or epimerization to form isopilocarpine.[65] In an attempt to study the influence of the carbamate nitrogen on physiological properties, certain carbamate analogues corresponding to pilocarpine were synthesized and evaluated.[66] Replacement of the carbon atom on the oxazolidinone ring with nitrogen resulted in the carbamate analogue **59** which was equipotent with pilocarpine (ED_{50} = 1 μM). This analogue bypasses the problem associated epimerization as observed in the case of pilocarpine. In addition, this cyclic carbamate would be expected to be less susceptible to hydrolysis than the lactone in pilocarpine.



58 **59**

Figure 40.

A more recent illustration of this replacement was observed in the evaluation of terbenzimidazoles as topoisomerase I inhibitors.[67] DNA topoisomerases are nuclear enzymes that control and modify the topological states of DNA by catalyzing the concerted breaking and rejoining of DNA strands.[68] Topoisomerases represent effective pharmacological targets for the development of cancer chemotherapeutics. Replacement of the phenyl (**60a**) with 2-pyridyl (**60b**), 3-pyridyl (**60c**), and 4-pyridyl (**60d**) resulted in retention of cytotoxicity and topoisomerase I inhibitory activity (Figure 41, Table 31).



60

Figure 41.

Table 31. Topoisomerase I-Mediated DNA Cleavage and Cytotoxicity of Terbenzimidazoles

compound	X	Topo I-mediated DNA cleavage[a]	cytotoxicity IC_{50} (μM) RPMI 8402
60a	phenyl	2	0.09
60b	2-pyridyl	3.3	0.16
60c	3-pyridyl	2	0.035
60d	4-pyridyl	2	0.035

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to Hoechst 33342, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of calf thymus topoisomerase I.

III. Nonclassical Bioisosteres

This major class of isosteres includes all those replacements that are not defined by the classical definitions of bioisosteres. These isosteres are capable of maintaining similar biological activity by mimicking the spatial arrangement, electronic properties, or some other physicochemical property of the molecule or functional group that is critical for retention of biological activity.

A. Cyclic vs Noncyclic Nonclassical Bioisosteric Replacements

This subclass includes all those nonclassical replacements wherein a noncyclic functional moiety mimics a cyclic group sterically or electronically resulting in retention of biological activity. Comparison of the various structural analogues of estradiol is a classical illustration of this phenomena. Here the ability of the structure to hold the critical functionality in a particular spatial configuration was essential for activity. It was found that the central bond of diethylstilbestrol (**2**, Figure 43) was important for the correct orientation of the phenolic and ethyl groups for binding to the estrogenic receptor.[69] This is evidenced by the observation that the *cis* isomer of **2** is only $^1/_{14}$ as active as the *trans* isomer.[70] Structurally or conformationally rigid analogues (compounds **2**−**4**) were equipotent as estradiol (**1**, Figure 43) when injected. Nonrigid analogues (compounds **5**−**7**), however, were found to have little or no estrogenic activity.[71,72]

This concept was extended by the use of the methyleneaminoxy methyl moiety (C=NOCH$_2$, MAOMM) (Figure 42) as a bioisostere of aryl and other aromatic groups.

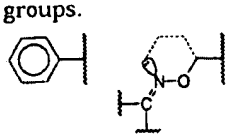


Figure 42.

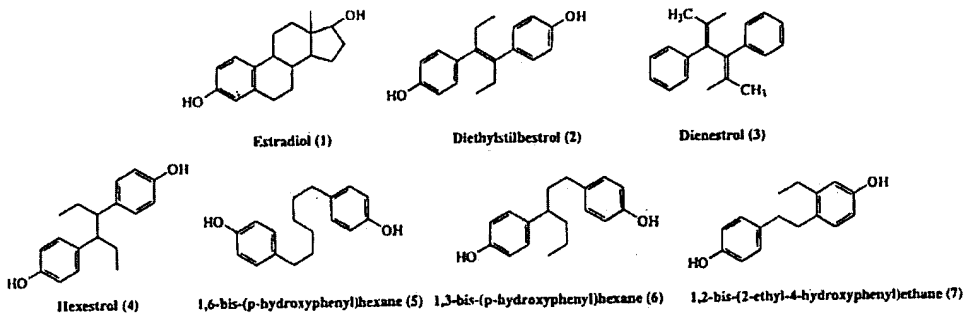


Figure 43.

Various studies associated with the development of β-adrenergic drugs have supported the utility of this nonclassical bioisostere.[73-75] Chemically synthesized analogues of biological catecholamines were among the first agents used to interact with β-adrenergic receptor sites. Analogues of the biological catecholamines often incorporated within their general structure a 1-phenyl-2-aminoethanol moiety (**8**, Figure 44). Closely related to these agents is a series of the 1-(aryloxy)-3-amino-2-propanol derivatives (**9**, Figure 44) which are β-adrenergic antagonists.[76-77] With regard to these adrenergic drugs, it has been observed that the aminoethanol portion, $-CH(OH)-CH_2NHR$ is present in both agonists and antagonists. Thus, it could be inferred from these data that this portion of these drug molecules plays a significant role in the ability of these compounds to bind with the receptor. On the other hand, it has been observed that the nature of the aromatic nucleus can significantly influence their agonistic or antagonistic properties. Results from theoretical studies[78,79] noted that the $C_3-O_2-C_4-C_5$ moiety of the class B type of β-blocking adrenergic drugs (**9**, Figure 44), can electronically and sterically simulate a portion of the aromatic ring. Data from these studies showed that the electronic distribution (based on the trend of the electrostatic molecular potential, EMP) generated by the $C_3-O_2-C_4-C_5$ was similar to that generated by the aromatic ring of antagonists such as **9**. This suggested that the electronic distribution essential for interaction with the β-adrenergic receptor may not require an aromatic ring.

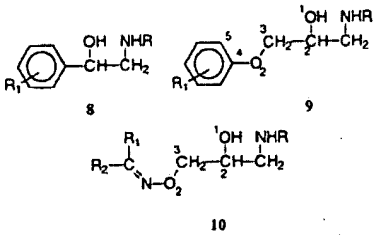


Figure 44.

These results led to the development of the aliphatic oxime ether derivatives (**10**, Figure 44) which showed marked and competitive antagonism at the β-adrenoceptors *in vitro* in pharmacological tests. This success led to the application of this bioisosteric replacement to other categories of drugs. One such application has been its use in the aryl acetic acids which are one of the most widely developed and investigated class of anti-inflammatory agents. Agents in this class include ibufenac (**11**), aclofenac (**12**), and diclofenac (**13**) which are widely used clinically (Figure 45).

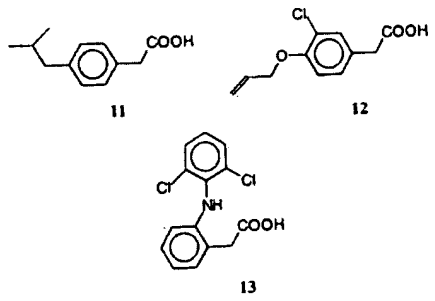


Figure 45.

Several β-aminooxypropionic acids were evaluated as analogues of arylacetic acids wherein the aryl moiety was substituted by MAOMM.[80] Of the various analogues evaluated, the (*E*)-3-[(benzylideneamino)oxy]propionic acid (**14**, Figure 46) showed similar potency to that of diclofenac (Table 32). The results of the conformational studies performed confirmed the existence of bioisosterism, at least from a steric point of view. Although the EMP trend, a measure of the electronic distribution, calculated for one of the β-aminooxypropionic acids and ibufenac showed that the MAOMM had a similar reactivity to that of the aryl moiety of ibufenac, it was observed in additional studies that this EMP trend was not essential for anti-inflammatory activity.

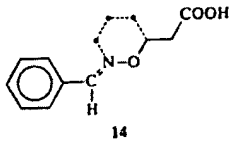


Figure 46.

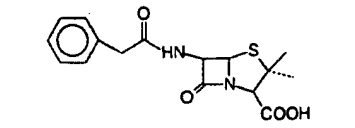
Table 32. Pharmacological Data of β-Aminooxypropionic Acid Derivatives

compound	% inhibition in the carrageenan paw edema assay
13 (Diclofenac)	67
14 (*E*)-3-[(benzylidene-amino)oxy]propionic acid	66

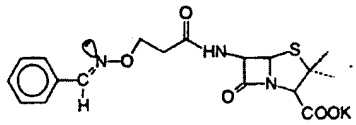
Further, as an extension to these studies, certain β-aminooxypropionyl penicillins and cephalosporin analogues were synthesized and evaluated for their antimicrobial properties.[81] It has been recognized that changes on the side chain linked to the β-lactam

nucleus of β-lactam antibiotics exert an influence on acid stability, resistance to enzyme inactivation, potency, and the spectrum of antimicrobial activity. It is observed among classical β-lactam antibiotics that those compounds widely used in therapeutic practice, such as ampicillin, cefalexin, and penicillin G (**15**), all have side chains of an acetamido type, substituted with an aromatic or a heteroaromatic ring.

A comparison of the MIC values outlined in Table 33 shows that replacement of the phenyl group of penicillin G (**15**) with a β-aminooxypropionyl group (**16**, Figure 47) results in retention of biological activity. The potency against both Gram-positive and Gram-negative bacteria, however, was reduced by approximately 50%.



15



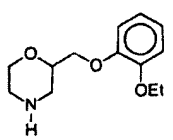
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Figure 47.

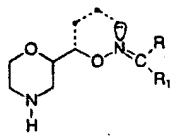
Table 33. Minimum Inhibitory Concentration (MIC) of β-Aminooxypropionyl Penicillins

	MIC (μg/mL)	
compound	Gram-positive bacteria	Gram-negative bacteria
15	0.05	71
16	0.10	136

Another recent application of this replacement has been documented for the morpholine class of antidepressants.[82] In this study, 2-[(methyleneamino)oxy]-methyl morpholines (**18**, Figure 48) were synthesized as analogues of viloxazine (**17**). Here the (aryloxy)-methyl group of viloxazine is substituted by a [(methyleneamino)oxy]methyl moiety (MAOMM). *In vivo* and *in vitro* tests showed that some of these morpholine derivatives possess a pharmacological profile similar to that of viloxazine (**17**).



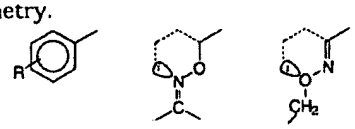
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18

Figure 48.

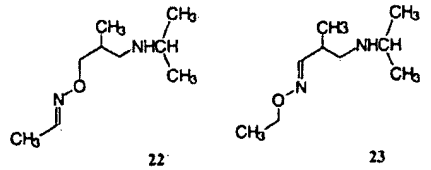
These earlier studies have shown that the [(methyleneamino)oxy]methyl moiety is a suitable bioisostere for the aryl moiety. It was noted however, that the carbon of the $-CH_2-$ linked to the oxygen (**20**, Figure 49) is of different hybridization (sp^3) than the corresponding aryl carbon (**19**, Figure 49) which is sp^2 hybridized. Further, in its preferred conformation, the unsaturated portion (C=N) of the MAOMM, is situated spatially in an area which does not exactly correspond to the area occupied by the aryl group. An attempt to optimize these differences resulted in the formal inversion of the atomic sequence $C{=}NOCH_2$ of the MAOMM (**20**, Figure 49) which lead to a different bioisostere, the [(methyloxy)imino]methyl moiety ($CH_2ON{=}C$, MOIMM) (**21**, Figure 49).[83] This moiety, in the *E* configuration, presents greater steric and electronic analogies to an aryl group as compared to MAOMM by virtue of similarities in hybridization and geometry.



19 20 21

Figure 49.

This similarity explains the retention of β_1-adrenergic binding affinity and activity (Table 34) on replacement of the MAOMM (**22**, Figure 50) with MOIMM (**23**, Figure 50). Results from the conformational studies and an analysis of molecular electrostatic potentials have shown a high degree of homology between these pharmacophoric groups and no significant differences in the chemical reactivity. Applications of this replacement (MOIMM) beyond this class of drugs have not yet been reported.



22 23

Figure 50.

Table 34. β-Adrenergic Activity and Radioligand Binding Affinity of MOIMM and MAOMM as Bioisosteric Replacements

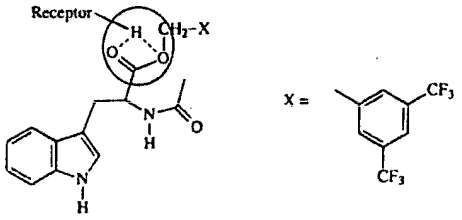
compound	β_1-adrenergic binding affinity[a] K_i (nM)	β_1-adrenergic activity[b] $-\log IC_{50}$
22	1480	4.52
23	1040	4.91

[a] β_1-Adrenergic binding affinity was measured using rat cortical membranes. [b] Isolated guinea pig atria was used to determine β_1-adrenergic activity.

A number of medicinal agents in clinical use contain an ester moiety as part of the molecule. The distribution of esterases in the body is ubiquitous and several types can be found in the blood, liver, and other organs and tissues. In many instances, the prevalence of these esterases causes these molecules to be highly labile *in vivo*. Bioisosteres of the ester moiety have been developed wherein such substitution can lead to an increase in the hydrolytic stability of the drug. One such replacement of the ester group is a heterocycle. The use of a ring in place of a noncyclic moiety is a common approach used to increase structural rigidity. When applied to ester moieties, such bioisosteric replacements can provide additional insights into the structural requirements of a specific receptor as well as result in the development of analogues with greater stability.

The role of the neuropeptide substance P in the endogenous response to pain and inflammation has

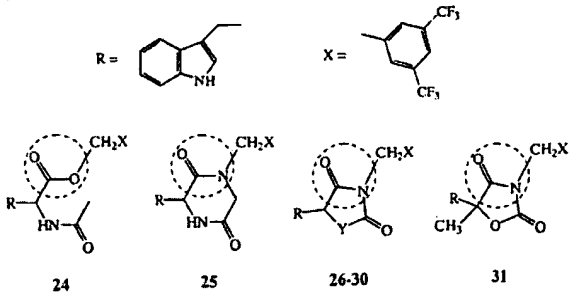
received considerable attention in recent years. These observations have suggested that suitable substance P receptor antagonists (NK_1 antagonists) may be of therapeutic use in the treatment of several clinical conditions including arthritis, migraine, postoperative pain, and nausea. (*S*)-Tryptophan benzyl esters such as L-732,138 (**24**, Figure 51) represent a new structural class of potent and selective NK_1 antagonists.[84] In this molecule the ester linkage is known to contribute significantly to receptor binding, presumably via hydrogen bonding.[85]



24, L-732,138

Figure 51.

A series of analogues in which 6-membered heterocyclic rings served as bioisosteric replacements for the ester linkage have been evaluated with the intent of improving their *in vivo* stability.[86] Although replacement of the ester by various substituted piperazines (**25**, Figure 52) and a variety of five-membered ring heterocycles (**26–30**) resulted in a loss of binding affinity (Table 35), the oxazolidinedione moiety (**31**, Figure 52) was identified as a useful bioisostere for the ester linkage and had the greatest potency with respect to NK_1 binding affinity. This analogue, however was less active than L-732,138 (**24**).



24 25 26-30 31

Figure 52.

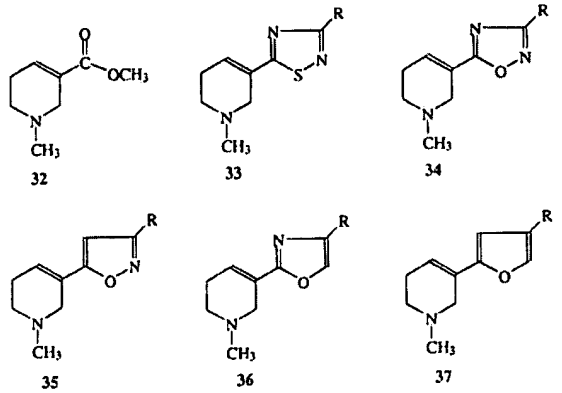
Table 35. NK_1 Antagonist Activity of Five- and Six-Membered Ring Heterocyclic Templates

bioisosteric ring (Figure 52)	Y	human NK_1 receptor binding affinity[a] (nM)
24 (L-732,138)	–	2.5
25	–	4333
26	NH	317
27	*N*-CH_3	153
28	*N*-$CONHCH_3$	63
29	CH_2	203
30	O	22
31	–	11

[a] Human NK_1 receptor binding affinity measured by displacement of [^{125}I]Tyr8-substance P.

Although this study failed to identify a more potent bioisostere because of the inability to attain optimal binding geometry, presumably the rings listed in Figure 52 could represent useful bioisosteric replacements for an ester moiety within another series of compounds. Unlike the ester moiety, these bioisosteres would not be subject *in vivo* to the action of esterases.

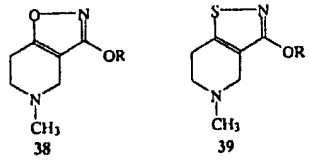
Another area in which bioisosteric replacements for the ester moiety have been investigated is in the design of centrally active muscarinic agents based on arecoline (**32**, Figure 53). Replacement of the ester group with either 3-alkyl-1,2,4-thiadiazoles (**33**) or 3-alkyl-1,2,4-oxadiazoles (**34**) have produced very potent muscarinic agonists.[87–92] Systematic removal of the heteroatoms in 3-methyl-1,2,4-oxadiazole gives isoxazoles and furans (**35–37**) with a decrease in affinity for the binding site. Thus, the electronic effects associated with these heterocyclic rings appear to be essential for muscarinic activity.



32 33 34 35 36 37

Figure 53.

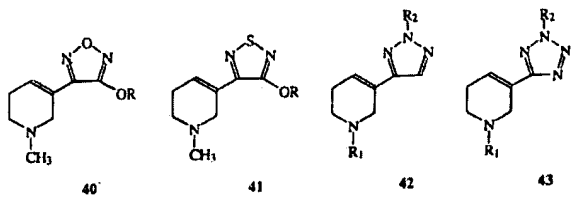
Certain other conformationally restricted compounds based on the bioisosteric replacement of the methyl ester groups with the 3-alkoxyisoxazoles (**38**) and the 3-alkoxyisothiazoles (**39**) (Figure 54) have also been found to result in muscarinic agonists with activity similar to arecoline.[93–94]



38 39

Figure 54.

On the basis of the structural resemblance of the 1,2,5-oxadiazoles and the 1,2,5-thiadiazoles with the 3-alkoxyisoxazoles (**38**) and the 3-alkoxyisothiazoles (**39**), several 1,2,5-oxadiazole (**40**, Figure 55) and 1,2,5-thiadiazole (**41**, Figure 55) analogues have been evaluated *in vitro* and found to be selective M_1 muscarinic agonists.[95] Recently a series of arecoline



40 41 42 43

Figure 55.

bioisosteres, where the ester group was replaced by a 1,2,3-triazol-4-yl (**42**) or a tetrazol-5-yl group (**43**), has been evaluated *in vitro* for affinity and efficacy at the muscarinic receptors and *in vivo* for cholinergic side effects.[96] The structure–activity relationships within these series are similar. Two compounds exihibited activity as M_1 agonists/M_2 antagonists, a profile of particular interest for drugs in the treatment in Alzheimer's disease. Further optimization showed that the position of the substituent in the 1,2,3-triazole or tetrazole ring has a significant influence on the affinity for muscarinic receptors. The 2-alkyl derivatives exerted high affinity, while 1- and 3-alkyl substituents resulted in analogues with very low affinity for the muscarinic recptors. This example clearly demonstrates the effective use of nonclassical bioisosterism in the development of new drugs which are significantly different from the lead compound.

Modulators of the central neurotransmitter GABA represent another class of compounds in which this concept of cyclic and noncyclic bioisosteres has played a major role. 4-Aminobutanoic acid (GABA, **44**, Figure 56) is the major inhibitory neurotransmitter in the mammalian central nervous system. This neurotransmitter functions at $GABA_A$, $GABA_B$, and probably $GABA_C$ receptors.[97–99] In order to pharmacologically characterize these receptors, a number of $GABA_A$ agonists bioisosterically derived from GABA, such as muscimol (**45**, Figure 56),[100–101] thiomuscimol (**46**, Figure 56),[101] and the much weaker $GABA_A$ agonist, isomuscimol (**47**, Figure 56),[101] have been developed. The establishment of specific $GABA_A$ agonists is of major therapeutic importance. It is anticipated that such research may represent the initial steps in the development of new types of antiepileptics, analgesics, and memory-improving drugs. The success of bioisosteric modifications in the development of GABA agonists is dependent on the ability of these receptors to tolerate bioisosteric modifications. Minor structural modifications in these agonistic molecules has frequently resulted in a marked or complete loss of activity.[102–103]

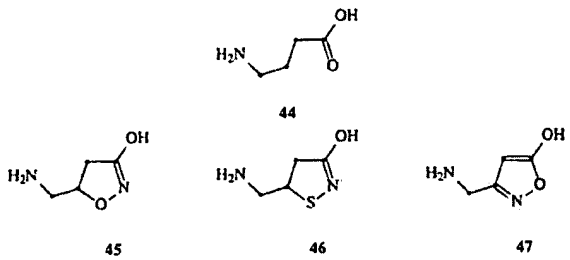


Figure 56.

The bioisosteric bicyclic analogues of these compounds, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, **48**, Figure 57),[101,104] 4,5,6,7-tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol (thio-THIP, **49**, Figure 57),[105] and 4,5,6,7-tetraisoxazolo[3,4-*c*]pyridin-3-ol (iso-THIP, **50**, Figure 57),[106] as well as isoguvacine (**51**, Figure 57),[101,104] and isonipecotic acid (**52**, Figure 57),[104] have been characterized as $GABA_A$ receptor ligands.

A further extension of the series of $GABA_A$ agonists described above led to another bicyclic series. On the basis of the lead that 5-(4-piperidyl)isoxazol-3-ol (4-PIOL, **53**, Figure 58) is a specific low-efficacy partial $GABA_A$ agonist,[107–109] several additional analogues of **53** were synthesized. The SARs for the 4-PIOL analogues showed no conspicuous relationships between pK_a values, receptor affinity, and agonist efficacy.[110]

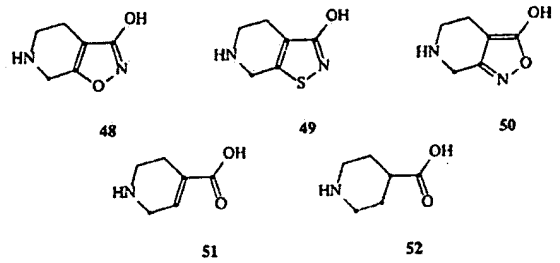


Figure 57.

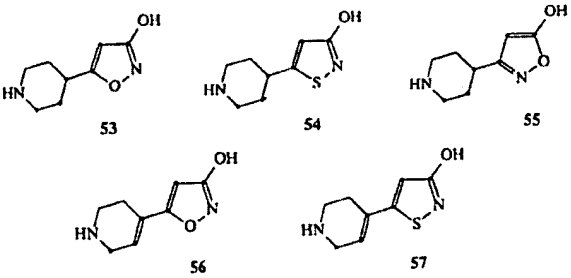


Figure 58.

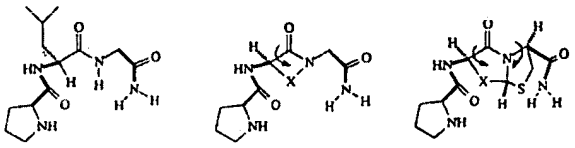
Although these bioisosteric replacements did not provide an analogue with substantially greater potency, these studies helped characterize several chemically distinct $GABA_A$ agonists which exhibited a range of efficacies. These bioisosteric replacements did provide insight into the spatial geometry required for these agents to maintain their specificity for a given pharmacological target.

The concept of cyclic and noncyclic bioisosteres has also been successfully applied to the development of several peptidomimetics. One of the most important aspects in the development of potent bioactive peptides and peptidomimetics is the process of elucidating the active conformation of the peptide. Advances in molecular biology have led to the identification and characterization of different subtypes of the same receptor, each of which are now known to be related to different physiological phenomena. The ability of a peptide to bind to a specific receptor subtype may require a particular conformation of the peptide. For this purpose, constrained peptidomimetics based on cyclic structures, constrained amino acids, and mimetics of peptide secondary structures have been used in efforts to duplicate the conformation that will bind to the specific receptor subtype and elicit a specific physiologic effect.

The tripeptide Pro-Leu-Gly-NH_2 (PLG, **58**) selectively enhances the binding of dopamine receptor agonists to dopamine receptors in the mammalian central nervous system.[111–113] Several lactam analogues **59**, where X was varied to give different sized or functionalized lactam rings, were synthesized and found to enhance the binding of the agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to dopamine receptors.[114,115] On the basis of this

study it was postulated that the bioactive conformation of PLG is a type-II β-turn. In order to test this hypothesis conformationally restricted bicyclic thiazolidine lactams (**60**, **61**, Figure 59), that restrict the torsion angles to values which are close to those of an ideal type-II β-turn, were synthesized and were more effective than PLG in enhancing the binding of the dopamine receptor agonist ADTN to dopamine receptors.[116]

This example demonstrates how cyclic bioisosteres could be used in place of noncyclic structures in peptides to restrict the torsion angles and evaluate the bioactive conformation essential for physiological activity.



58 59 60 X = CH_2 61 X = CH_2CH_2

Figure 59.

B. Nonclassical Bioisosteric Replacements of Functional Groups

In this section possible nonclassical bioisosteric replacements for different functional groups will be discussed. As can be the case for any bioisostere, not all of these replacements will necessarily result in a compound with comparable biological activity to the template drug. However, in those instances where such replacements have resulted in retention of biological activity, the examples as outlined in this section may encourage the use of these isosteres in future structure activity studies.

1. *Hydroxyl Group Bioisosteres*

Nonclassical bioisosteres for phenolic hydroxyl groups generally do not resemble this functional group in terms of size or potential as a strong electron-donating group. Several nonclassical bioisosteres for the phenolic hydroxyl group are listed in Table 36. Molar refractivity is an index of relative size. While the hydroxymethyl bioisostere most closely approximates the size of the phenolic hydroxyl group, it remains significantly different. In terms of these nonclassical bioisosteres, only the urea bioisostere is an electron-donating substituent. Thus, these nonclassical bioisosteres are unlikely to be suitable in those instances where biological activity is adversely affected by increased molecular size or is strongly dependent on electronic parameters. These nonclassical bioisosteres tend to be most effective, in those instances where the role of the phenolic hydroxyl group is to act as either a hydrogen bond acceptor or donor. Such bioisosteres are also effective when moderate hydrophilicity is correlated with improved biological activity.

Table 36. Aromatic Substituent Constants for Hydroxyl Group Bioisosteres[117]

bioisosteres	σ_p	π	MR
OH	−0.37	−0.67	2.85
CH_2OH	0.00	−1.03	7.19
$NHCONH_2$	−0.24	−1.30	13.72
$NHCOCH_3$	0.00	−0.97	14.93
$NHSO_2CH_3$	0.03	−1.18	18.17
NHCN	0.06	−0.26	10.14

A widely used nonclassical bioisosteric replacement for the hydroxyl group is the alkylsulfonamido group. The acidity of the exchangeable proton of the phenolic hydroxyl group and an alkylsulfonamide is comparable. Further, the −N−H portion of the sulfonamide is able to align itself, in relation to the receptor, in a manner closely approximating the phenyl −O−H grouping (Figure 60). An illustration of the successful use of this replacement is in its use in a series of compounds that possess a similar biological profile to that of structurally related phenolic ethanolamines.[118]

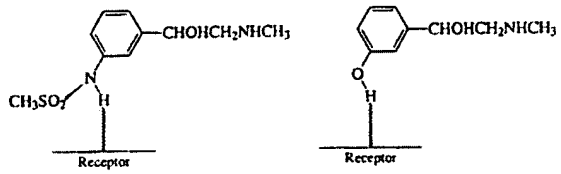


Figure 60.

Notable examples of β-adrenoceptor agonists in which a 3-hydroxyl group has been replaced with bioisosteric groups (Figure 61) include albuterol (**63**, 3-CH_2OH), soterenol[119] (**64**, 3-$NHSO_2CH_3$), and carbuterol[120] (**65**, 3-$NHCONH_2$). Isoproterenol (**62**, Figure 61), the prototype of the β-adrenergic agonists, is widely used clinically as a bronchodilator; however, it is not a β_2-selective agent. In addition this catechol is rapidly metabolized by catechol *O*-methyl transferase (COMT) which catalyzes the methylation of the *m*-hydroxy group.[121] Attempts to increase the duration of action of this class of agents by preventing this route of metabolism has resulted in the replacement of the *m*-hydroxyl with bioisosteres such as the methanesulfonamido, hydroxymethyl, and ureido groups, resulting in agents with potent and selective activities (Figure 61).

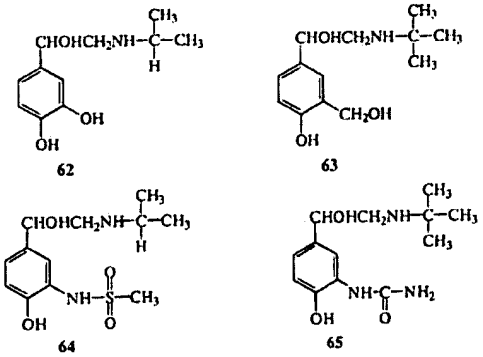
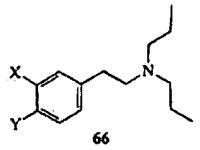


Figure 61.

A more recent illustration of bioisosteric replacements for a phenol was observed in a study designed to evaluate the D_1- and D_2-receptor affinity for a series of *N,N*-di-*n*-propyldopamine (DPDA) analogues (Figure 62).[122] In this study, a number of analogues in which a hydroxyl group was substituted with nonclassical bioisosteric replacements were evaluated



66

Figure 62.

Table 37. Ligand Binding Data for *N,N*-Di-*n*-propyldopamine (DPDA) Cogeners

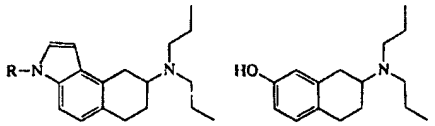
compound	X	Y	Hill coefficient, pIC_{50}[a]
66a	OH (DPDA)	OH	7.65
66b	NHCHO	OH	6.94
66c	$NHCOCH_3$	OH	5.10
66d	$NHCONH_2$	OH	5.15
66e	$NHSO_2CH_3$	OH	7.87
66f	$NHCOCH_3$	H	4.69
66g	$NHCONH_2$	H	4.87
66h	$NHSO_2CH_3$	H	5.45

[a] Negative logarithm of the concentration required to produce 50% inhibition in EDTA-washed rat strital membranes using [^{3}H]spiperone

(Table 37) for their ability to bind to the D_2-receptor in rat striatal membranes.

In this illustration, it is evident (Table 37) that the methanesulfonamide (**66e**) appears to be a suitable bioisosteric replacement for the 3-hydroxyl group of DPDA. The formamide group (**66b**) also serves as a bioisostere in this series, but this analogue exhibited lower affinity for the D_2-receptor than DPDA. Both of these bioisosterically derived compounds also demonstrated *in vivo* cardiovascular and renal profiles that were consistent with selective D_2-receptor agonism.

Certain nitrogen heterocycles can serve as potential bioisosteres for the phenolic moiety. Heterocycles, such as pyrrole, indole or benzimidazoles, that have a proton attached to a nitrogen atom and whose lone pair of electrons is involved in maintaining aromaticity, have proved particularly effective. Benz[*e*]-indole (**67**, Figure 63), the pyrrolo analogue of the dopaminergic agonist (**69**),[123-125] displays potent dopaminergic properties, is orally active and has a

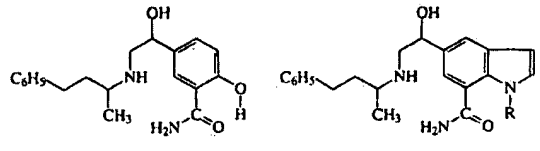


67 R = H
68 R = CH_3 69

Figure 63.

longer duration of action than **69**. The effectiveness of the pyrrolo ring of **67** as a bioisosteric replacement for the phenolic hydroxyl group has been ascribed to the ability of both groups to hydrogen bond to a common acceptor nucleus on the dopamine receptor.[126] Evidence for the involvement of the hydrogen bond formation being related to the success of this bioisosteric replacement was provided by the lack of activity observed with the *N*-methylpyrrolo analogue **68**.

Examination of the antihypertensive activity of the pyrrolo analogue **71** of labetalol (**70**, Figure 64), an α- and β-adrenergic receptor antagonist[127-128] provides further evidence for the successful use of the indole phenol bioisosteric replacements.[129]

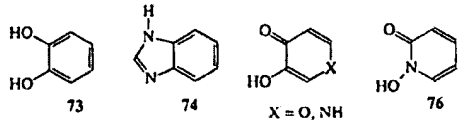


70 71 R = H
72 R = CH_3

Figure 64.

The indolo analogue **71** of labetalol (**70**) was found to reduce blood pressure in spontaneously hypertensive rats, without causing a decrease in heart rate. The antihypertensive activity of these compounds is related to their ability to hydrogen bond to the adrenergic receptors. The importance of a hydrogen atom on the indole nitrogen atom is again evidenced by the fact that the N-methylated analogue **72** is inactive. While both **70** and **71** are antihypertensive agents, there are differences between these molecules with respect to their lipophilicities and pK_a values. While the phenol derivative is acidic, the indole analogue is almost neutral. These differences may partially explain the subtle differences in pharmacological activity between these compounds. Labetalol, in contrast to **71**, shows no significant β_1-ISA and therefore exhibits negative chronotropic activity.

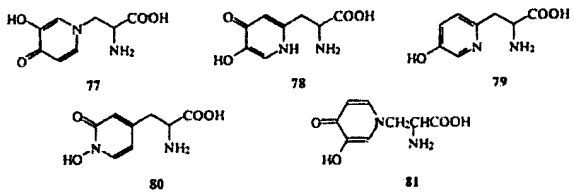
Bioisosteres have also been developed as replacements of both hydroxyl groups of catechols. Several examples are illustrated in Figure 65.[3]



73 74 X = O, NH 75 76

Figure 65.

The success of L-dopa in the treatment of parkinsonism resulted in the search for analogues of dihydroxyphenylalanine which would exhibit fewer side effects. Some of these hydroxypyridine and pyridine-quinoid-type compounds are listed in Figure 66.[130-133]



77 78 79 80 81

Figure 66.

2. Carbonyl Group Bioisosteres

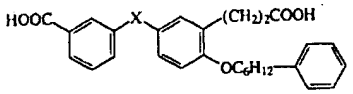
Carbonyl group bioisosteres for the purposes of this review refer to replacements which have been used primarily for the aldehyde or ketone moiety (Table 38). The difference in the electronegativity between oxygen and carbon associated with these functional groups results in a partial positive charge on the carbon atom while the oxygen acquires a partial negative charge. Comparison of the substituent

constants associated with isosteres for the carbonyl group illustrate that these nonclassical bioisosteric replacements are generally electron-withdrawing moieties that are relatively large in size.

Table 38. Aromatic Substituent Constants for the Carbonyl Group Bioisosteres[134]

bioisosteres	σ_p	π	MR
$SOCH_3$	0.49	−1.58	13.70
SO_2CH_3	0.72	−1.63	13.49
CH=NOH	0.10	−0.38	10.28
$CH=NOCH_3$	0.30	0.40	14.93

In the section on monovalent isosteres (hydroxyl–thiol interchanges), the pharmacological basis leading to the development of different classes of antagonists of Leukotriene B_4 (LTB_4) was discussed. LTB_4 has been implicated as a potential mediator of inflammation. Elevated amounts of LTB_4 have been found in human psoriatic plaque[135–136] and in the colonic mucosa of patients with inflammatory bowel disease.[137–138] Levels of LTB_4 in these tissues appear to correlate with the extent of disease. The design of potent and selective antagonists of LTB_4 has been proposed for the development of new therapeutic approaches for the treatment of these diseases or any condition where LTB_4 may play a role as a pathological mediator. A series of benzophenone dicarboxylic acids (Figure 67) were synthesized as potential inhibitors of LTB_4 to its receptors in intact human neutrophils.[139] Replacement of the carbonyl with a variety of polar and nonpolar bioisosteres led to marginal changes in binding affinity (Table 39).



82

Figure 67.

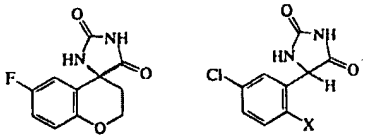
Table 39. Receptor Affinity of LTB_4 Receptor Antagonists with Modified Linking Groups

compound	X	percent inhibition of specific [^{3}H]LTB_4 binding (μM)
82a	C=O	85
82b	C=NOH	84
82c	(C=O)NH	73
82d	S	67
82e	SO	71
82f	SO_2	79

It was observed that the oxime (**82b**, Table 39) showed activity most similar to that of the corresponding benzophenone (**82a**, Table 39). The activity of the amide-linked (**82c**, Table 39) and the sulfur-linked derivatives (**82d–f**, Table 39) was also similar to that of the benzophenone. While the sulfoxides and sulfones are recognized as nonclassical bioisosteres suitable for replacement of a carbonyl group, thioethers such as **82d** are not. However, the lack of any significant difference in activity upon replacement of the carbonyl with either a thioether, sulfoxide or sulfone, which differ widely with respect to their polarities and hybridization, suggests that this portion of the molecule is not critically involved in LTB_4 receptor binding. This example illustrates how bioisosteric analogues can be used to identify those sites on the drug which have a major impact on the interaction that occurs with the pharmacophore.

The sulfoxide and the sulfone moieties have increasingly been used as nonclassical divalent bioisosteres of the carbonyl group.[140–145] In addition, metabolic oxidation of a pharmacologically active agent which has a thioether incorporated within their structure results in the formation of corresponding sulfoxide and sulfone analogues. In many instances, these are more potent than the corresponding sulfides. The greater size associated with the sulfone moiety has also been shown to be a factor that modulates biological activity. This was illustrated in the development of novel aldose reductase inhibitors related to sorbinil (**83**, Figure 68).

Sorbinil is a spirocyclic hydantoin that exhibits activity as a potent inhibitor of the enzyme aldose reductase.[146] On the basis of this property, a number of spirohydantoin aldose reductase inhibitors have been studied.[147] In an attempt to simulate the rigid nature of a spirocyclic hydantoin with a nonspirocyclic compound several analogues (**84**, Figure 68) with different substitutions at the 2-position were synthesized and evaluated for their ability to inhibit aldose reductase.[144] Comparison of their IC_{50} values in Table 40 reveals that the sulfone analogue is the most potent analogue, with significantly greater activity than the thioether and sulfoxide analogues. The sulfone moiety in this instance hinders the rotation of the hydantoin ring, forcing it to be aligned in a manner similar to that of the spirocyclic hydantoin sorbinil.



83 84

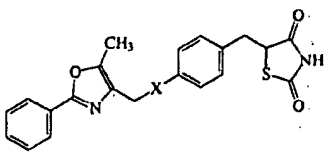
Figure 68.

Table 40. Biological Activity of Nonspirocyclic Hydantoin Aldose Reductase Inhibitors

compound	X	IC_{50}[a] (μM)
83	Sorbinil	0.12
84a	SO_2CH_3	0.05
84b	$SOCH_3$	0.25
84c	SCH_3	0.62

[a] Concentration that causes a 50% inhibition of human placental aldose reductase with glyceraldehyde as substrate.

Another example of the use of carbonyl bioisosteres is illustrated for a novel series of thiazolidine-2,4-diones that were evaluated as potent euglycemic agents. Non-insulin-dependent diabetes mellitus (NIDDM) is a metabolic disorder characterized by hyperglycemia as well as insulin resistance and/or impaired insulin secretion.[148,149] Most commmonly used agents in the therapy of NIDDM are the sulfonylureas.[150,151] CP-86,325 (**85a**, Figure 69) is representative of a relatively new class of glucose-lowering agents.[152] Several bioisosteres in which the ketone moiety of CP-86,325 has been replaced, have been synthesized and evaluated for their ability as glucose-lowering agents (Table 41). While these



85

Figure 69.

Table 41. Euglycemic Activity of Various Ketone Isosteres

compound	X	dose (mg/kg)	% glucose normalization in ob/ob mouse[a]
85a	$CH_2C{=}O$ (CP-86.325)	1	100
85b	CH_2SO_2	5	69
85c	CONH	5	64
85d	$CH_2C(NOH)$	5	100
85e	$CH_2C(NOMe)$	5	100

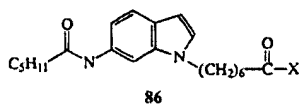
[a] Ciglitazone was dosed at 50 mg/kg as a positive control and results are reported as the percentage of glucose normalization compared to the standard ciglitazone-treated group (100% at 50 mg/kg) and the vehicle treated group (0%).

bioisosteres did retain activity as euglycemic agents, none of these analogues exhibited potency similar to **85a**[153] (Table 41).

3. Carboxylate Group Bioisosteres

Nonclassical bioisoteres for the carboxylate group consist of replacements which involve (a) only the hydroxyl portion or (b) both the hydroxy and carbonyl fragments of this functional group.

a. Replacements Involving Only the Hydroxyl Portion. The types of nonclassical bioisosteres used for the replacement of the acidic hydroxyl group of a particular carboxylic acid are similar to the nonclassical bioisosteres which have been previously outlined for phenolic hydroxyl groups. This subclass of bioisosteric replacements used in place of the carboxylate group will, therefore, not be reviewed in detail. The determination of suitable replacements for the carboxylate group is often based on the ability of the bioisostere to possess similar acidity and to exhibit similar physicochemical properties. Replacement of the hydroxyl moiety of a carboxylic acid with a phenyl sulfonamide, for example, results in the formation of a sulfonimide. The estimated pK_a values for sulfonimides are similar to that of an aryl carboxylic acid. A recent example of the use of this bioisoteric replacement is in the development of indole derived leukotriene antagonists (Figure 70).[154] As indicated in Table 42, there is little difference between the sulfonimide derivative **86b** and its parent compound



86

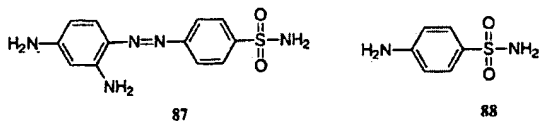
Figure 70.

Table 42. Biological Activity of 7-[6-Hexanamidoindol-1-yl]heptane Derivatives

compound	X	% antagonism of 8 nM LTE_4-induced contraction
86a	OH	53
86b	$NHSO_2Ph$	58

86a with respect to their ability to antagonize LTE_4-induced contraction.

b. Replacements Involving the Entire Carboxylate Functional Group. In 1932, it was discovered that Prontosil (**87**, Figure 71) was able to provide cures of streptococcal infections in mice.[155] The active form of Prontosil was later proven to be *p*-aminosulfanilamide (**88**).[156]



87 88

Figure 71.

The mechanistic basis for the antimicrobial activity of sulfonamides is their ability to act as competitive inhibitors of the incorporation of *p*-aminobenzoic acid associated with the formation of dihydropteroic acid, thereby, ultimately inhibiting the biosynthesis of dihydrofolic acid (Figure 72).[157] Thus, one of the earliest synthetic antibiotics developed consisted of an antimetabolite in which a nonclassical bioisostere replaced a carboxylic acid moiety.

While sulfonamides represent one of the first nonclassical bioisosteres used to replace the carboxyl group, there has been increased interest in the use of tetrazole as a nonclassical bioisosteric replacement for carboxylate groups. Table 43 outlines a comparison of the physicochemical properties of the carboxyl group and its anionic derivative with those of the tetrazole in both its neutral and anionic state. Replacement of the carboxylic acid moiety with a tetrazole is well known and has increasingly been used with success within different classes of medicinal agents. Comparison of these groups at physiological pH reveals that the tetrazole group is almost 10 times more lipophilic while having similar acidity, $pK_a = 4.9$, to that observed for carboxylic acids (pK_a 4.2–4.4).

Table 43. Aromatic Substituent Constants for Carboxylic Acid Bioisosteres[117]

bioisosteres	σ_p	π	MR
	0.45	−0.32	6.9
	0.00	−4.36	6.0
	0.56	−0.48	15.6
	0.35	−3.55	14.6

A recent study of second generation benzodiazepine CCK-B (cholecystokinin) antagonists (**89**, Figure 73) can serve as an illustration of the bioisosteric replacement of the carboxylate group with a tetrazole moiety resulting in enhanced potency due to the reduced hydrophilicity.[158] The effect of increasing lipophilicity, upon replacing a carboxylic acid with tetrazole, on CCK-B receptor binding can be seen in Table 44. Further replacement with an oxadiazolone ring in place of the carboxylic acid resulted in even

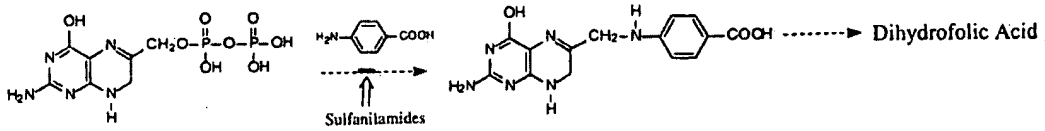
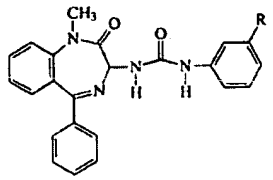


Figure 72.

greater potency at the CCK-B receptor (Table 44). The greater activity of tetrazole and the oxadiazole is attributed to the optimal placement of the polar substituent facilitating a favorable interaction within the CCK-B receptor.



89

Figure 73.

Table 44. Receptor Binding Affinities and Solubility Properties 3-(Phenylureido)-1,4-benzodiazepines

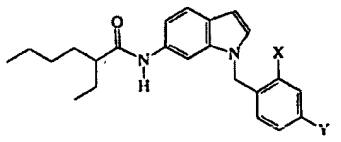
R	IC_{50}[a] (nM) CCK-B	solubility, mg/mL
	6.69	4.7
	1.02	3.74
	0.266	0.41

[a] Concentration of compound required for half-maximal inhibition of the binding of [^{125}I]BH CCK-8s to receptors in guinea pig cortical membranes.

Other commonly used isosteres for the carboxylic acid moiety, besides sulfonamides and tetrazoles, include sulfonate and phosphate. Among these bioisosteric replacements, both the sulfonates and phosphates are more hydrophilic than the carboxylate anion and are 100% ionized at physiological pH.

To illustrate the applicability of these replacements, the literature outlines a systematic structure–activity study of the carboxylic acid region in a series of indole- or indazole-derived leukotriene antagonists (**90**, Figure 74).[154] The peptidoleukotrienes are known to be the active components of the "slow reacting substance of anaphylaxis" (SRS-A).[159] SRS-A is believed to be an important biological mediator in several disorders, especially human allergic diseases. Thus, the design and development of selective antagonists of leukotrienes presents a potential target for the treatment of asthma.

In a series of leukotriene antagonists (Table 45), it was observed that the sulfonic acid analogue, (**90b**) was equipotent with the carboxylic acid (**90a**). Bioisosteric replacement with the sulfonimide moiety, which has a pK_a similar to that of the carboxylic acid,[160] resulted in a 100-fold increase in activity. Transposition of the carbonyl and sulfonyl groups resulted in a loss of activity which was similar to that of a carboxylic acid. The spatial relationship between the pendant phenyl group and the sulfone was noted as being responsible for the increased potency of analogue **90c** as compared to analogue **90d** (Table 45).



90

Figure 74.

Table 45. Biological Activity of Indole Acid Leukotriene Antagonists

compound	X	Y	test concentration (10^{-7} M)	% antagonism of LTE_4
90a	H	COOH	100	44
90b	H	SO_3H	100	48
90c	H	$CONHSO_2Ph$	1	42
90d	H	$SO_2NHCOPh$	100	37

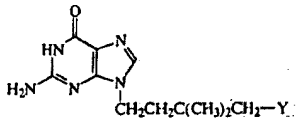
Table 46. LTE_4 Antagonism of Indole Acid Leukotriene Antagonists

compound	X	Y	test concentration (10^{-7} M)	% antagonism of LTE_4
90e	OCH_3	COOH	10	59
90f	OCH_3	1*H*-tetrazol-5-yl	10	50
90g	OCH_3	$CONHSO_2CH_3$	100	100
90h	OCH_3	$CONHSO_2Ph$	1	100

Among the methoxy analogues (**90e–h**, Table 46), it was also observed that the phenyl sulfonimide (**90h**) was 100 times more potent than the methylsulfonamide (**90g**). In this study, the tetrazole analogue was also shown to be equipotent to the carboxylic acid. The methylsulfonimide, however, was only one-tenth as active as the carboxylic acid derivative (**90e**, Table 46). The pK_a value of the methylsulfonamide is similar to that of the phenylsulfonamide and the carboxylic acid.[160] The increased potency of the phenylsulfonamide compared to the methylsulfonamide in this instance was attributed to a region of the receptor which specifically binds the phenyl of the sulfonamide (**90h**).

The phosphate, as previously noted, can be viewed as being interchangeable with the carboxylate moiety and other related bioisosteres. In a recent study, a number of functional end groups attached to the 9-position of guanine by an alkyl side chain (Figure 75), were evaluated as phosphate mimics on the basis of their ability to inhibit purine nucleoside phosphorylase (PNP).[161] This provided a sensitive method for the evaluation of the ability of these groups to occupy the phosphate binding site of PNP.

The data obtained (Table 47) reveal that the sulfonic acid interacts quite well with the phosphate binding site, while the carboxyl group does not



91

Figure 75.

Table 47. Inhibition of Purine Nucleoside Phosphorylase by Guanine Analogues

compound	Y	IC_{50} (μM)[a]
91a	PO_3H_2	0.04
91b	SO_3H	0.18
91c	SO_2NH_2	100.0
91d	COOH	8.0
91e	$CONH_2$	200.0

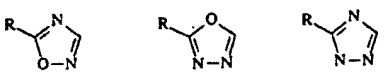
[a] Concentration required to cause 50% inhibition of calf spleen PNP.

interact as well. The sulfonamide (**91c**) and the carboxamide (**91e**), however, have little affinity for this binding site. X-ray crystallography studies show that the binding of the sulfonic acid analogue is almost identical to that of the phosphate analogues, as illustrated by the IC_{50} values. The loss of activity by substitution of the phosphate with the carboxy group was specifically related to the hydrogen bonding of the carboxyl group with the hydroxyl of serine 220 and the amide nitrogen atom of methionine 219 of PNP. These interactions pull the guanine moiety out of the position it normally occupies in the purine binding site of PNP.

4. *Amide Group Bioisosteres*

Bioisosteric replacements for the amide represents an area that is currently the center of focus because of its implications in peptide chemistry and the development of peptide mimetics. Peptide bonds and peptide fragments have been replaced with a wide variety of structural moieties in attempts to convert peptides into chemically stable and orally available molecules. In view of the fact that replacements for the peptide bonds have been reviewed extensively,[162-163] only some of the bioisosteric replacements of the amide will be discussed.

Esters have been widely used as replacements for amides. In the section on cyclic vs noncyclic bioisosteres, the use of heterocyclic rings as replacements for the ester moiety has been illustrated. Heterocyclic rings such as 1,2,4-oxadiazoles (**92**),[164-168] 1,3,4-oxadiazoles (**93**),[169-171] and triazoles, such as the 1,2,4-triazoles (**94**)[172] (Figure 76), have also been used as replacements for amide or ester bonds.

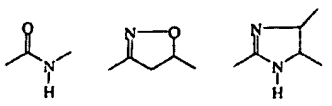


92 93 94

Figure 76.

Some of the other heterocyclic rings used in peptidomimetic chemistry include the 2-isoxazoline[173] (**96**, Figure 77) and imidazoline moieties[174] (**97**, Figure 77).

Another approach that has facilitated the design and development of HIV protease inhibitors has been



95 96 97

Figure 77.

the application of the "transition-state peptidomimetic" principle. This principle involves the replacement of the hydrolyzable peptide linkage with nonhydrolyzable transition-state isosteres.[175] Figure 79 outlines the hydrolysis of a peptide bond with its transition-state intermediate. A series of transition-state mimetics of the peptide bond is illustrated in Figure 78.

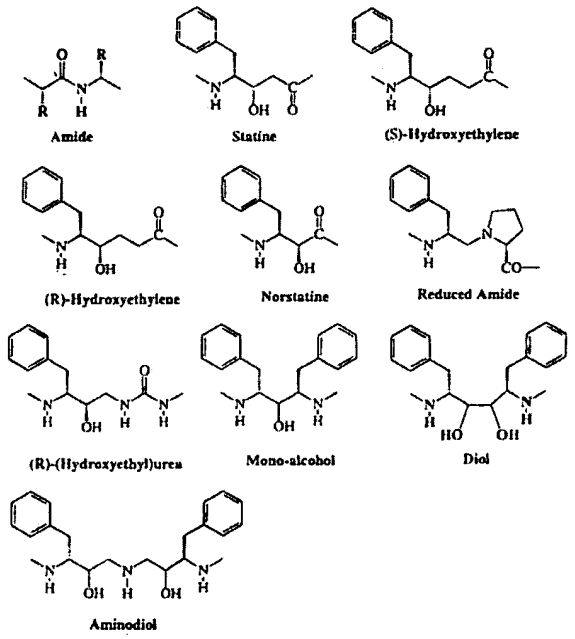


Figure 78.

Listed in Table 48 are possible bioisosteric replacements for the amide bond. The ability of these groups to be suitable bioisosteres depends on the role the amide group plays in eliciting the biological activity and the ability of the bioisostere to mimic this role as closely as possible. Two examples will be discussed, one in which the hydrogen donor and acceptor functionalities of an amide are required for maintaining biological activity and another in which the amide group functions only as a spacer between two functional groups.

Table 48. Bioisosteres of the Amide Bond

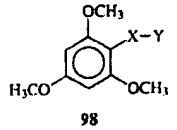
bioisostere	formula
amide	$-NHCO-$
reversed amide	$-CONH-$
thioamide	$-NHCS-$
amide homologue	$-CH_2NHCO-$
ketomethylene	$-COCH_2-$
urea	$-NHCONH-$
methyleneamino	$-CH_2NH-$
carbamate	$-NHCO_2-$
thiocarbamate	$-NHCOS-$
ester	$-CO_2-$
sulfonamide	$-NHSO_2-$
hydroxyethylene	$-CH(OH)CH_2-$



Figure 79.

The enzyme acyl-coenzyme A:cholesterol acyltransferase (ACAT) catalyzes the formation of cholesteryl esters from cholesterol and fatty acyl-CoA. Accumulation of cholesterol and its esters in coronary arteries is a prominent feature observed in atherosclerotic patients. Further evidence suggests that ACAT is necessary for hepatic very low density lipoprotein secretion[176] and intestinal absorption of cholesterol.[177] Thus, inhibition of ACAT may be of therapeutic value in the treatment of atherosclerosis by reducing the amount of cholesterol absorbed in the intestine, reducing VLDL secretion, and retarding the accumulation of cholesteryl esters in the artery walls.[177] In an attempt to define the structural features for potent ACAT inhibition, a series of oleic and fatty acid analogues were synthesized with various bioisosteric replacements of the amide group. These were evaluated for their ability to inhibit ACAT *in vitro* (Table 49).[178]

Among compounds **98e–h** listed in Table 49 (Figure 80), it was observed that only the urea bioisostere **98e** retained ACAT inhibitory activity *in vitro*. Apparently, bioisosteric substitutions having both hydrogen bond donor and acceptor functionalities were required to maintain potent inhibitory activity as illustrated by the thioamide (**98d**), carbamate (**98f**), urea (**98e**), and thiocarbamates (**98g**) in Table 49. The location of the hydrogen bond donor and acceptor was important for activity since the reversed amide (**98b**) was less potent than the amide.



98

Figure 80.

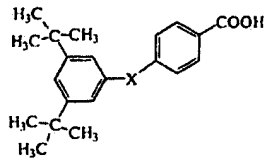
Table 49. *In Vitro* Activity of Bioisosteric Analogues of 2,4,6-Trimethoxy Anilides of Oleic and Stearic Acid

compound	X	Y	IC_{50}[a] (μM)
98a	NHCO	$C_{17}H_{33}$	0.044
98b	CONH	$C_{18}H_{35}$	9.4
98c	CH_2NH	$C_{18}H_{35}$	>100
98d	NHCS	$C_{17}H_{33}$	0.9
98e	NHCONH	*n*-$C_{18}H_{37}$	0.9
98f	$NHCO_2$	*n*-$C_{18}H_{37}$	7.1
98g	NHCOS	*n*-$C_{18}H_{37}$	5.7
98h	$CH_2NHCONH$	*n*-$C_{18}H_{37}$	>5

[a] ACAT inhibition *in vitro* in intestinal microsomes isolated from cholesterol-fed rabbits.

In contrast to the previous example, the main function of the amide group in retinobenzoic acids (**99**, Figure 81) appears to be in the positioning or spacing of the *m*-dialkylphenyl and the *p*-carboxyphenyl group.[179]

It was observed that amide (–NHCO–) group replacements[179,180] such as –CONH–,[180,181] $-SO_2NH-$, –COCH=CH–,[182,183] –N=N–[184,185] retained activity, regardless of their electronic properties or ability to act as hydrogen bond donors.

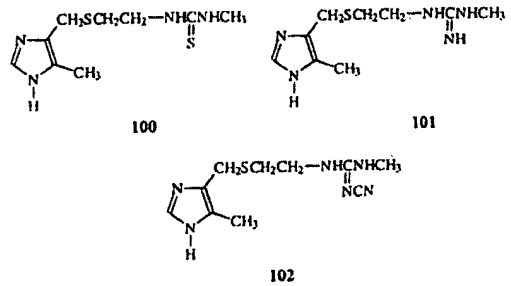


99

Figure 81.

5. *Thiourea Bioisosteres*

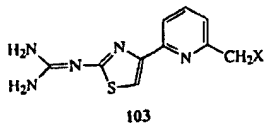
Thiourea bioisosteres have been successfully employed in the development of H_2-receptor antagonists. Histamine H_2-receptor antagonists have been widely used in the treatment of peptic ulcer disease. Side effects such as agranulocytosis observed in early clinical studies with the H_2-antagonist metiamide (**100**, Figure 82) was attributed to the thiourea moiety. Replacement with a guanidino group (**101**, Figure 82) resulted in absorption problems because of its high degree of ionization at physiological pH. Further, bioisosteric substitution with the cyanoguanidino derivative provided cimetidine (**102**, Figure 82), which was twice as active as metiamide as an inhibitor of gastric acid secretion. The presence of a polar nonbasic functional group attached to the secondary position of an (ethylthio)methyl substituent on an aromatic heterocycle exists for all four of the major H_2-antagonists which are in clinical use. These include cimetidine, famotidine, ranitidine, and nizatidine.



100 101 102

Figure 82.

In the continuing search for novel H_2-antagonists, a series of 2-guanidino-4-pyridylthiazole derivatives (**103**, Figure 83) were synthesized and evaluated for gastric antisecretory activity.[186] Replacement of the cyano group in the cyanoguanidine moiety with electron-withdrawing groups such as a sulfonylalkyl group or nitromethine resulted in retention of activity. However these analogues were less potent than cimetidine (Table 50).



103

Figure 83.

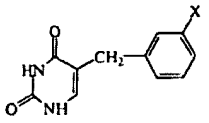
Table 50. Pharmacological Activities of 2-Guanidino-4-pyridylthiazoles

X	% inhibition of gastric secretion[a]
cimetidine	53
$NHC(=NCN)NHCH_3$	15
$NHC(=NSO_2CH_3)NHCH_3$	24
$NHC(=CHNO_2)NHCH_3$	18

[a] Inhibition of histamine-stimulated gastric acid secretion in the lumen-perfused stomachs of the anesthetized rats.

6. Halogen Bioisosteres

The last class of isosteres which will be discussed are the isosteres for the halogens. Halogens have been replaced by electron-withdrawing groups such as a cyano or trifluoromethyl group. Replacements of this type were observed in a series of 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils (**104**, Figure 84) that were tested for inhibition of liver uridine phosphorylase (UrdPase).[187] Uridine phosphorylase is an enzyme that catalyzes the reversible phosphorolysis of pyrimidine nucleosides. Uridine phosphorylase is responsible for the degradation of chemotherapeutic agents such as 5-fluoro-2'-deoxyuridylic acid.[188] Thus, the development of agents belonging to this class has been of chemotherapeutic interest.[189] Within the series of 5-benzyluracils, it was suggested that electron-withdrawing groups at the 3-position decreased potency. This hypothesis was supported by the observation that replacement of the chloro atom with stronger electron-withdrawing groups such as the cyano or the triflurometh resulted in less potent analogues (Table 51).



104

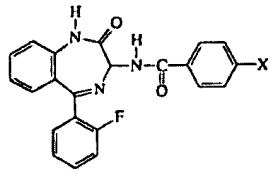
Figure 84.

Table 51. Uridine Phosphorylase Inhibition of 5-Benzyluracils

compound	X	IC_{50}[a] (μM)
104a	Cl	2.5
104b	CN	13.2
104c	CF_3	21.4

[a] Concentration of inhibitor that results in 50% decrease in reaction velocity.

Another illustration of such nonclassical replacements for halogen was illustrated in a structure–activity relationship study of cholescystokinin-A receptor antagonists (**105**, Figure 85).[190] In the section on carboxylate bioisosteres the pharmacological basis behind the design of CCK-A receptor antagonists has been discussed. Here again CCK receptor antagonists have been proposed as potentially useful in the treatment of appetite disorders, abnormalities of gastric motility, biliary tract disease, pain management, and psychiatric disorders.[191] As illustrated in Table 52 replacement with nonclassical bioisosteres of the halogens in the benzodiazepines resulted in retention of antagonistic activity at the CCK-A receptor.



105

Figure 85.

Table 52. Pancreas Receptor Binding Affinities for 3-(Benzoylamino)benzodiazepines

compound	X	$-\log(IC_{50})$[a]
105a	Cl	8.54
105b	CN	7.37
105c	CF_3	7.70

[a] Micromolar concentration of maximal inhibition of binding of [^{125}I]CCK-33 or [^{125}I]CCK-8 to rat pancreas.

IV. Conclusion

Bioisosteres modulate biological activity by virtue of subtle differences in their physicochemical properties. Systematic correlation of physicochemical parameters with observed biological activity has been very effective in highlighting subtle differences within bioisosteric groups which often increase activity. Of significance is the ability of these bioisosteric groups to define some of the essential requirements of the pharmacophore. This is especially important when the synthesis of a large number of drug candidates for evaluation is not economically feasible. A number of less known replacements have not been reviewed because of their inability to demonstrate bioisosterism in more than a single class of agents. In this review, an attempt has been made to explain the rationale behind the use of bioisosteric replacements using examples from current literature. It is hoped that this systematic approach will facilitate the use of bioisosteric replacements in future structure–activity studies.

V. Acknowledgments

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INFLUENCE OF ISOSTERIC REPLACEMENTS UPON BIOLOGICAL ACTIVITY

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PART 1

INTRODUCTION

To the synthetic organic chemist interested in medicinal chemistry, every physiologically active compound of known structure is a challenge - a challenge either to better it, or perhaps merely to equal it. For it must be remembered that even the most innocuous drug is not tolerated by some people.

There are numerous ways of attacking such a problem, and this audience is certainly familiar with them. One of the methods which has been used frequently, very often with success, is that of isosteric replacement. The examples of this type of replacement in the literature are very numerous, and the fruitful results in the fields of sulfonamides, antimetabolites, and antihistamines are well known.

The concept of isosterism, first introduced by Langmuir,[13] has been changed over the years by the work of many others. It will be the object of this paper to survey the history of isosterism, to classify the varieties of isosteric replacements which are recorded in the literature, and to note the influence of these replacements on the biological activity of compounds. We shall then be able to see if any general relationships apply, and what conclusions may be drawn from such data.

PART 2

THEORETICAL

Langmuir in 1919[13] pointed out the remarkably similar physical properties of carbon dioxide and nitrous oxide. He deduced from the octet theory that the number and arrangement of electrons in these molecules are the same. Compounds showing such relationship to one another were termed isosteric compounds or isosteres. These terms were not restricted to compounds, but were extended to groups of atoms which hold pairs of electrons in common (termed by Langmuir comolecules). Comolecules were likewise considered isosteric if they contained the same number of atoms and possessed the same number and arrangement of electrons.

Langmuir predicted twenty-one types of isosteric groups of which only a few will be mentioned here:

CHART 1

Langmuir Type	
2	$O^{=}$, F^{-}, Ne, Na^{+}, Mg^{++}, Al^{+++}
3	$S^{=}$, Cl^{-}, A, K^{+}, Ca^{++}
5	Br^{-}, Kr, Rb^{+}, Sr^{++}
8	N_2, CO, CN^{-}
9	CH_4, NH_4^{+}
10	CO_2, N_2O, N_3^{-}, CNO^{-}

Langmuir postulated that when isosteric comolecules are also isoelectric, that is when they have the same total charge, all their physical properties should be closely similar. Only three such pairs occurred in Langmuir's tables:

N_2 and CO; CO_2 and N_2O; N_3^{-} and NCO^{-}

However, no direct comparison can be made of the physical properties of isosteres having different electrical charges.

Even though the classes are distinct from one another, it was demonstrated that comparisons of comolecules in different groups could still be made. If any two substances are very much alike in physical properties, then any isoelectric isosteres of these substances should show close relationships with one another. Thus in types 3 and 8, argon and nitrogen resemble each other closely, therefore chloride ion (isosteric with argon) should resemble the cyanide ion (isosteric with nitrogen). Likewise the similarity between K^{+} and NH_4^{+} can be derived from argon and methane. It is to be emphasized that in Langmuir's terminology K^{+} and NH_4^{+} are not isosteres of one another.

Whereas Langmuir compared only physical properties, Seifriz[17] showed in 1948 that CO_2 and N_2O are both reversibly anesthetic to the slime mold Physarum polycephalum.

In 1921, W. Huckel[11] pointed out that the imino group (=NH) in homopolar compounds corresponds to the oxygen atom and that the $-NH_2$ and -OH groups correspond to the F atom.

Somewhat later (1925) Grimm[8] very markedly extended the concept of isosterism. In place of Langmuir's term "isosteric comolecule", Grimm preferred the term pseudoatom. His hydride displacement law states:

> "Atoms anywhere up to four places in the periodic system before an inert gas change their properties by uniting with 1 to 4 hydrogen atoms, in such manner that the resulting combinations behave like pseudoatoms, which are similar to elements in the groups 1 to 4 places, respectively, to their right."

Grimm showed this relationship by the following chart:

CHART 2

	Group	4	5	6	7	0	1
Number of	0	C	N	O	F	Ne	Na
Hydrogens	1		CH	NH	OH	FH	
	2			CH_2	NH_2	OH_2	FH_2^+
	3				CH_3	NH_3	OH_3^+
	4					CH_4	NH_4^+

Beginning in 1932, Professor Erlenmeyer[5] at the University of Basel in Switzerland has published a series of papers on isosteric compounds. He has given great impetus to the modern concept of isosterism in organic chemistry, particularly in relation to biological activity. Erlenmeyer accepted Grimm's classification and has broadened it even further. His definition of isostere is:

> "Atoms, ions, or molecules in which the peripheral layers of electrons can be considered to be identical are termed isosteres."

By Erlenmeyer's definition all elements in the same group of the periodic table are isosteric so long as they have the same number of electrons in their outermost shell. In a unique application of this concept, Erlenmeyer in 1933[6] considered S and CH=CH in an aromatic nucleus to be isosteric by counting only the "peripheral" electrons in the C=C pseudoatom (whether it be written -CH=CH- or =CH-CH=).

In 1946, Mentzer[14] demonstrated that, in certain circumstances, the group $-CH_2-CH_2-$ could be replaced by -CO-O- with no change in biological activity of the parent compound. He did not term these isosteric pairs.

Some authors, as Mentzer[14] and Erlenmeyer[3] use the term potential-cycle or pseudocycle to bring out the steric relationships between the ring and opened form of physiologically active molecules.

Occasionally the term "isolog" is used by some authors (as Fieser[7] in the United States and Steinkopf[18] in Europe) where "isostere" is usually designated. Isologous compounds, however, need not be isosteric.

It is obvious from this brief survey that the term "isosteric" has varied in meaning with different writers - from a narrow to a very broad concept. In this discussion we shall accept the term in its broadest meaning and study the influence of isosteric replacements on the biological activity of molecules. We shall not consider physical properties, although it is not implied that such properties as mixed-crystal formation are not of great significance for isosteric relationships.

We shall term compounds "bio-isosteric" if they fit the broadest definition for isosteres and have the same type of biological activity.

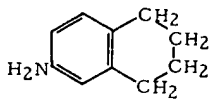
The biological equivalence of isosteric groups receives support from immunological studies. Landsteiner[12] was able to prepare artificial antigen-antibody systems by coupling diazotized aromatic amines with proteins, and injecting these protein complexes into animals to form antibodies. He discovered that these antibodies have the specific power of combining with the group attached by the azo linkage. This group, which is of known structure, he termed the haptenic group. The specificity of the antibody in any serum could be tested, by means of the precipitin reaction, with related complex proteins. In general, the combining power is highly,

but not completely, specific.

Erlenmeyer,[4,19] using
several isosteric atoms and ps
isosteric replacement, where

C_6H_5-O-C_6H_4-N=N-Prc
C_6H_5-NH-C_6H_4-Protein
C_6H_5-CH_2-C_6H_4-Protei
-C_6H_4-SO_3H
-C_6H_4-SeO_3H
not -C_6H_4-SO_2H

I



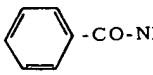
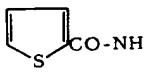
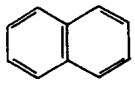
I and II	+++
I and III	±
II and III	++

From Erlenmeyer[4,19]

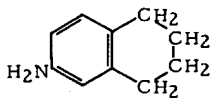
but not completely, specific.

Erlenmeyer,[4,19] using Landsteiner's method, demonstrated the serological similarity of several isosteric atoms and pseudoatoms. The following are illustrations of several types of isosteric replacement, where the resulting compounds give cross-reactions:

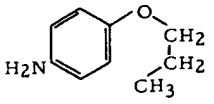
CHART 3

SEROLOGICAL SPECIFICITY	
C_6H_5-O-C_6H_4-N=N-Protein C_6H_5-NH-C_6H_4-Protein C_6H_5-CH_2-C_6H_4-Protein	-C_6H_4-PO_3H_2 -C_6H_4-AsO_3H_2 not -C_6H_4-SbO_3H_2
-C_6H_4-SO_3H -C_6H_4-SeO_3H not -C_6H_4-SO_2H	 -CO-NH-C_6H_4-  -CO-NH-C_6H_4- not  -CO-NH-C_6H_4-

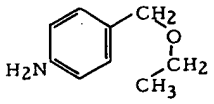
I



II



III



I and II +++

I and III ±

II and III ++

From Erlenmeyer[4,19]

While this work is not necessarily foolproof, and indeed has been criticized by Heidelberger[10] as requiring more and better support, it is in good agreement with Pauling's views.

Pauling[15] has also studied serological reactions to provide information about the molecular structure and configuration of simple substances. He compared the process of antibody formation to the production of a replica by pressing a plastic material against a mold and permitting it to harden. The polypeptide chain, with its power of assuming alternative configurations, is the plastic material, and the surface of the antigen serves as the die or mold. The complementariness of antigen and antibody includes not only surface configuration, but also juxtaposition of special combining groups, such as a negatively charged group in the antibody with a positively charged group in the antigen, and a hydrogen-bond-forming group carrying the proton with a similar group presenting an electron pair. Thus, isosteric replacements in an antigen which do not affect the shape or polarity of the molecule should not interfere with its reaction with the antibody.

Pauling has extended this concept of spatial surface configuration to include biological specificity in general.

Deductions from serological reactions are limited because strongly polar groups have predominant effects. However, this type of study should show when parts of a molecule are simple "space fillers", that is occupy specified geometrical bulk.

Since the discovery that the antagonism of the sulfonamides to p-amino benzoic acid is an antimetabolite effect, due to the close similarity of structure, isosteric replacements in other essential nutrients have yielded many compounds of interest. This field has been thoroughly reviewed in the literature,[16] and is the subject of a separate part of the symposium. We shall merely mention some of the types of isosteric replacement in the numerous antimetabolites which have been made, and only a few specific examples will be given in this paper:

CHART 4

Essential Nutrient	Atom or Group Replaced	Replacement
Riboflavin	2-CH_3	2-Cl
Thymine	-CH_3	-OH, -Br, or -NH_2
Mesoinositol	6-OH	6-Cl
Thymine, lysine, folic acid	-NH_2	-OH
Folic acid	-OH	-NH_2
p-Amino benzoic acid, glutamic acid	-COOH	-$CONH_2$
p-Amino benzoic acid, niacin	-COOH	-$COCH_3$
Arginine	-O-	-CH_2-
Uracil, thymine, niacin amide	-O-	-S-
Methionine	-S-	-O-, or -CH_2-
Purines	-CH=	-N=
Phenyl alanine, p-aminobenzoic acid	benzene	pyridine

Essential Nu
Phenyl alanine
Niacin
Thiamine
Methionine
Valine, niacin, pantc aspartic acid, oxyb
p-Amino benzoic aci
p-Amino benzoic aci

The isosteric compound fo
usually it may have an anta
gives compounds acting by

Ideally, to make co
necessary: (1) Substances
involved in the test should
for many types of biologica
tests, we cannot be sure th
chosen which are based on
organism. It is not claime
clinical studies; nonethele
adaptable to other series of

Activities found in o
compounds in another test.
those in which the authors
lists.

Biological activities
side effects of drugs, will

CHART 4 (Cont.)

Essential Nutrient	Atom or Group Replaced	Replacement
Phenyl alanine	benzene	thiophene, furan, pyrrole
Niacin	pyridine	thiazole
Thiamine	thiazole	pyridine
Methionine	-S-	-CH=CH-
Valine, niacin, pantothenic acid, aspartic acid, oxybiotin, heteroauxin	-COOH	$-SO_3H$
p-Amino benzoic acid	-COOH	$-AsO_3H_2$
p-Amino benzoic acid	-COOH	$-PO_3H_2$

The isosteric compound formed may have either the same activity as the original, or more usually it may have an antagonistic effect. In either case, it is proof that isosteric replacement gives compounds acting by the same mechanism, that they are truly bio-isosteric.

Ideally, to make comparisons between structure and biological activity, two criteria are necessary: (1) Substances compared must act by the same mechanism and (2) The structure involved in the test should be the structure of the compound under study. However, in practice, for many types of biological activity only in vivo tests can be used, and even when using in vitro tests, we cannot be sure that the above criteria apply. In so far as possible, examples have been chosen which are based on in vitro activity, and mainly those using an isolated tissue or micro-organism. It is not claimed that the in vitro tests will necessarily correlate with in vivo or clinical studies; nonetheless the data obtained may be a useful guide for further work and may be adaptable to other series of compounds.

Activities found in one screening test need not parallel the relative activities of the compounds in another test. Since data in the literature are usually lacking for tests other than those in which the authors were most interested, it is seldom possible to make such alternate lists.

Biological activities, as absorption, distribution, conjugation (detoxification), taste, odor, side effects of drugs, will not be discussed.

PART 3

TABLES OF DATA

In order to classify the data for presentation, the following chart is pertinent for organic compounds.

CHART 5

Class 4	Class 3	Class 2	Class 1	
	Sb	Te	I	
	As	Se	Br	
	P	S	Cl	
C	N	O	F	Ne
N^+ P^+ S^+	-CH-	-NH- -PH-	-OH -SH	
		$-CH_2-$	$-NH_2$ $-PH_2$	
			$-CH_3$	

Table modified from Grimm[8]

We have designated these four types from the number of covalent bonds. Above are the elements of the same periodic group, below are the isosteric hydrides. The following tables will show the effect of isosteric replacement on biological activity within each type. The -S- and -C=C-, and other special cases do not fit into this chart and will be treated separately.

Discussion of Tables

Class 1 - Halogens and Hydrides (OH, NH_2, CH_3)

Tables 1-4 list examples of comparisons of Class 1 of the chart previously shown. Table 1 contains examples of multiple comparisons, Table 2 compares halogens only, Table 3 compares halogens with hydrides, Table 4 compares hydride with hydride. An attempt has been made to select examples from fields of current interest.

Table 1. If one were to judge results of isosteric replacements from Table 1, it would be difficult to arouse enthusiasm. The most unusual case is the replacement of the chain -OH of epinephrine by NH_2; the activities of other members of this series should be of great theoretical interest.

Table 2. In general the results are what would be expected, activities usually showing a gradient with the molecular weights. There are exceptions, mostly with the extreme members F and I.

Tables 3 and 4. These again show unpredictability of response. The α-hydroxy-β-phenylethylamine examples have been amply discussed by Hartung.[9]

General Conclusions to Class 1. It is not possible to predict when members of this class will be bio-isosteric; in most instances they will not be. Very often activity is specific to one member which would be called, in Ehrlich's terminology, an anchoring group. Differences in activities may be attributable to differences in polarity of the groups, to solubility differences, or to chemical reactivity. The most likely pairs of bio-isosteres are:

halogen and CH_3

halogen and OH,

the most unlikely pairs are:

OH and NH_2.

Class 2 - (S, O, NH, CH_2)

Tables 5 - 7 inclusive list examples of Class 2 replacements. For convenience, the examples have been divided into structural types: ether, ketone, and ester.

Table 5: Ether Type. These show a better probability of bio-isosterism than Class 1 types. The member which fits in the least is -NH. Sulfur is not always bio-isosteric with oxygen, in fact surprisingly less than might be anticipated. Probably polarity differences play a predominant role.

Table 6: Ketone Type. The most interesting examples are probably the thio-barbiturates. In general, these types have restricted comparability.

Table 7: Ester Type. Many amides and thioesters related to the local anesthetic and antispasmodic esters are known, but practically none have come into use. More thought should be given to the replacement of ester oxygen by the CH_2 group.

General Conclusions to Class 2. Isosteric replacement in this group has better promise of usefulness than in Class 1. While methoxy and ethyl often do not show similarity, in other cases interchanging O and CH_2 yields compounds of similar activity. Here, as in Class 1, polarities probably play a dominant role.

Class 3 - Tertiary N and Tertiary C

Tables 8 and 9 list examples of Class 3. Most of the known examples of this class occur in the aromatic ring systems (Table 8). No attempt has been made to list the numerous examples in well-known fields as the sulfonamides and antihistamines but a few are given to refresh memories. Commercially this has been the most valuable application of bio-isosterism.

Table 9 contains non-aromatic examples. Because of polarity differences the aliphatic types can seldom be expected to display bio-isosterism, but it is a more likely assumption that diphenylamine and benzhydryl derivatives would show such similarity. More examples are desirable.

While tertiary P, As and Sb theoretically are electronically isosteric with N and CH, practically, except between As and Sb, they show little bio-isosterism.

Class 4 - Quaternary C, N, S, Etc. (Table 10)

The spatial tetrahedral geometry and the positive charge are of paramount importance for this class. In general, a quaternary carbon, because it lacks a charge, is not interchangeable with quaternary nitrogen.

The Special Classes

Table 11 - Aromatic C=C and O, S, NH. Following Erlenmeyer, it is generally agreed that the pairs, benzene and thiophene, and pyridine and thiazole, are isosteric. Furan and pyrrole differ markedly from benzene in both physical and chemical properties. Indeed, Bradlow, Vanderwerf and Kleinberg[2] in a brief discussion of the concept of isosterism, state that "Proponents of the principle of isosterism do not point out the fact that by definition pyrrole is also isosteric with benzene, thiophene and furan." There are sufficient examples, however, to indicate that these rings may be bio-isosteric although the furan and pyrrole compounds are usually weaker in activity than those containing benzene and thiophene. The activity of furan isosteres in the antihistamine field indicates that such replacements cannot be ignored.

The latter part of the table contains comparisons of an ethylenic bridge between two aromatic rings with S, O and NH. Not enough examples are available to draw conclusions, but this should be an interesting replacement type.

Table 12 - Carbonyl and Sulfone (or Sulfoxide). The structural relationship between p-aminobenzoic acid and sulfanilamide has been emphasized by Bell and Roblin[1] in explaining the mode of action of sulfonamides. The exchange of -COOH for $-SO_3H$ in many metabolites to produce antagonistic substances has shown this to be a general phenomenon. Therefore, a comparison has been made in this table of compounds with carbonyl and sulfone groups. The sulfoxide has been added since spatially it more nearly resembles the carbonyl than does the sulfone grouping. Electronically neither the sulfoxide nor the sulfone group is isosteric with the carbonyl group. The ionic bond of the sulfur groups further emphasizes the difference.

The table does not indicate any striking resemblances except for the amidone type example.

Table 13 - $-CO-O-$ and $-CH_2-CH_2-$. These groups are not electronically isosteric; it is most likely that they owe their activity to similar spatial fixation (as discussed in the following type).

Table 14 - Spatial Fixation by Ring or Double Bond. That three dimensional spatial characteristics play a highly important role in biological activity is known to everyone. The vastly different activities often noted in optical or cis-trans isomers must be considered in any attempt to explain the mechanism of biological activity. When two molecules are almost identical spatially, that is, are superimposable in three dimensions, we may expect similar activity provided the polarities are situated in corresponding parts of the molecules.

Table 14 lists examples of ring and open forms. In many cases there is striking agreement; often however, one form is completely inactive. Where agreement between activities is found, it is interesting to assume that the open and closed forms can be superimposed in three dimensions.

Benadryl is an interesting example. When the rings are forced into the planar form of the fluorene ring, activity is lost; one might speculate therefore that in the "active" form of Benadryl the two benzene rings do not lie in the same plane. From the example of Trasentin and Pavatrine the opposite appears to be the case.

Table 15.- Polarity Shift, Exo-Endo Cyclic. This and the following table are offered in an attempt to systematize data scattered throughout the literature. This table demonstrates the effect of moving a polarity from without to within a ring, the shift being to the adjacent position. The probability is that the polarity of the atom must remain quite similar in order to retain the same activity.

Table 16 - Reversed Adjacent Polarity. This table illustrates the effect of reversing adjacent polar groups. Many instances are of great interest; this is a transformation to be kept in mind in seeking new compounds.

We have seen that
ment - isosteres need not
simplest living systems.

As we all know, si
In addition, there are two
One is that we discover wl
(i.e., the anchoring group
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The similarity of t
already achieved through i
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CONCLUSIONS

We have seen that similarity in biological action need not result from isosteric replacement - isosteres need not be bioisosteres. This is not surprising in view of the complexity of the simplest living systems.

As we all know, simple isosteric replacements often give compounds of interest and value. In addition, there are two important types of information to be learned from such replacements. One is that we discover which groups cannot be eliminated in order to retain the desired activity (i.e., the anchoring groups); the other is that we learn which parts of the molecule are important because of their bulk space characteristics. These facts enable a more intelligent approach to the synthesis of new compounds.

The similarity of biological activity in so many instances, and the successful results already achieved through isosteric replacements, show that this is a type of variation which the synthetic chemist must keep in mind. If chemical reactivity and polarities are considered, the predictability of bio-isosteric replacement is quite high.

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EXPLANATION OF TABLES

In the first column the structural formulas of the compounds under consideration are written with the variable isosteric group represented as **X**.

In the second column the biological list is stated (e.g. antitubercular, narcotic, anesthetic, etc.) together with other pertinent data necessary - as to whether in vitro or in vivo, what organ, organism, or animal was used, what challenging agent, if any, was used, and in what terms the activity data are expressed with a designation of the reference compound if such was used. Below, in parenthesis, the references to the literature are given. This list is found immediately following the tables.

The remaining column headings usually designate the atom or group represented by **X**. In cases where this is not so, the heading is self evident. The data in these columns are the activities of the compounds in the terms used by the original workers; this varies with different authors from quantitative figures, a system of plusses, to mere statements of activity or non-activity.

TABLE 1

CLASS 1

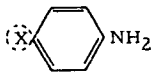
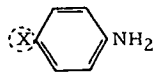
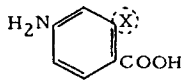
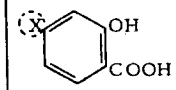
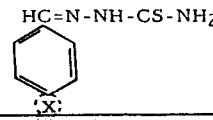
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Anti-tubercular In vitro Salicylate No. (16)	F < 1 Cl 6 Br 7 I 20	< 1	< 1	27
	Anti-tubercular % curative in guinea pigs (111)	Cl 19%	16%	-	inactive
	Anti-tubercular In vitro (94)	Cl inactive	most active	inactive	weak
	Anti-tubercular In vitro Molar conc. for stasis (72)	Cl 1/500	1/1,000	1/40,000	
	Anti-tubercular In vivo (mouse) (74)	Cl slight	definite	slight	

TABLE 1 (Cont.)

CLASS 1

Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Phenol Coefficient vs. B. Thyphosus	F 1 Cl 3.9	12	?	2.5

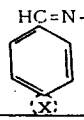
	Molar conc. for stasis (72)				
	Anti-tubercular In vivo (mouse) (74)	Cl slight	definite	slight	

TABLE 1 (Cont.)

CLASS 1

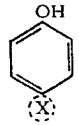
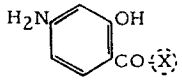
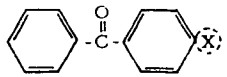
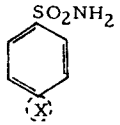
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Phenol Coefficient vs. B. Thyphosus Phenol = 1 (140)	F 1 Cl 3.9 Br 5.4	12	?	2.5
	Anti-tubercular In vitro Molar Conc. for Stasis (72)		1/40,000	1/80	1/17
	Anti-tubercular In vitro Stasis - mg % (57)	Cl 2.5	10		2
	Antibacterial (120)	inactive	inactive	active	inactive

TABLE 1 (Cont.)

CLASS 1

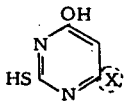
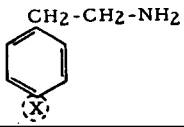
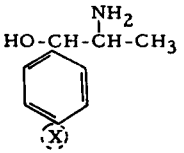
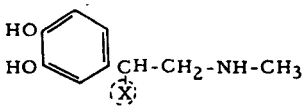
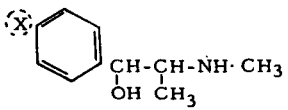
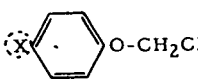
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Antithyroid (109,138)		inactive	weak	potent
	Pressor Activity Epinephrine = 1 (66,103)	F or Cl "weak"	1/70		"weak"
	Pressor Activity (66,143,64,81,67)	F, Cl "possess pressor activity"	1/50 epi	? activity unpublished	1/200 epi
	Pressor Activity (18,152,93)	Cl - known ?	OH - epi SH - "epi-like"	1/10 epi	?
	Pressor Ephedrine = 1 (66)		< 1	2	< 1

TABLE 1 (Cont.)

CLASS 1

Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Muscle relaxant Ø-O-CH_2-CHOH-CH_2OH = 1	Cl 2.1		0.2	1.2

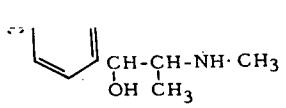
	Ephedrine = 1 (66)				

TABLE 1 (Cont.)

CLASS 1

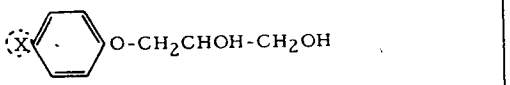
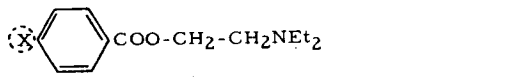
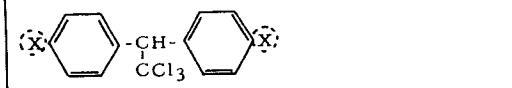
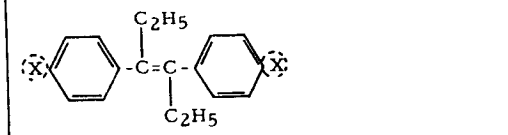
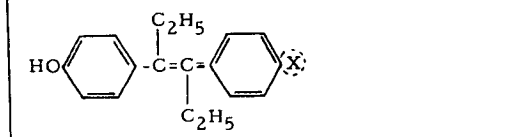
Compound	Biological Test (Reference)	Halogen	-OH	$-NH_2$	$-CH_3$
	Muscle relaxant Ø-O-CH_2-CHOH-CH_2OH = 1 (99)	Cl 2.1		0.2	1.2
	Local anesthetic Procaine = Standard (27,128)	F comparable	comparable	(standard)	
	Insecticidal vs. Lice Standard = DDT (113)	F 1/4 DDT Cl DDT Br 1/2 DDT	inactive	inactive?	1/5 DDT
	Estrogenic (135)	Br very weak	potent 0.4 gamma	very weak	inactive?
	Estrogenic (135)	Br 100 gamma	0.4 gamma	7.5 gamma	

TABLE 1 (Cont.)

CLASS 1

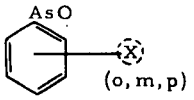
Compound	Biological Test (Reference)		Halogen	-OH	$-NH_2$	$-CH_3$
	vs. T. pallidum		Cl			
	in vitro	ortho	83	84	88	84
		meta	110	79	104	97
	Ø AsO = 100	para	85	72	83	102
	(38)					

TABLE 2

CLASS 1

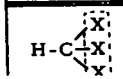
Compound	Biological Test (Reference)	I	Br	Cl	F
	Narcosis	narcotic	narcotic	narcotic	inactive

TABLE 2

CLASS 1

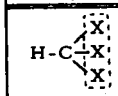
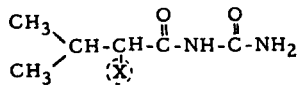
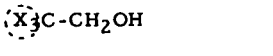
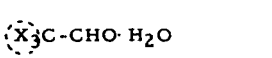
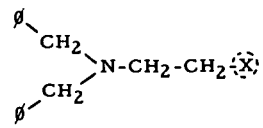
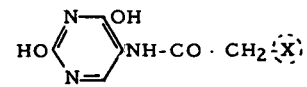
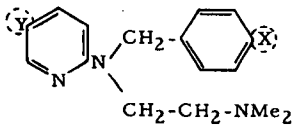
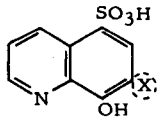
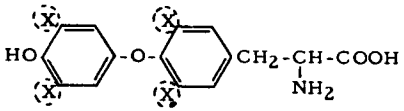
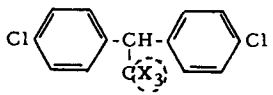
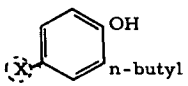
Compound	Biological Test (Reference)	I	Br	Cl	F
	Narcosis (56, 70)	narcotic	narcotic	narcotic	inactive
	Hypnosis (56)	inactive	hypnotic	hypnotic	
 	Narcosis (91)		narcotic (2 x Cl cmpd) inactive	narcotic narcotic	
	Adrenergic blocking (119)		active (same as Cl)	active	
	vs. Vaccinia virus (144)			active	inactive

TABLE 2 (Cont.)

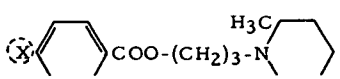
CLASS 1

Compound	Biological Test (Reference)	I	Br	Cl	F
	Antihistamine X PBZ = 1 Y (147)	- 1/2 -	1 1/2	2 1/2	3
	Amebic Dysentery (149)	active	inactive	inactive	
	vs. Myxedema in humans (95)	1	1/17	1/250	
	Insecticidal (113)		good	excellent	weak
	Phenol coefficient vs. S. aureus (140)		313	257	60

314

TABLE 3

CLASS 1

Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Local anesthesia minutes	12	15	10			?	9

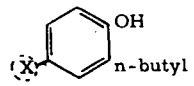
	Phenol coefficient vs. S. aureus (140)			313	257	60		

TABLE 3

CLASS 1

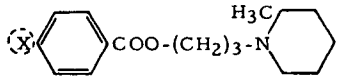
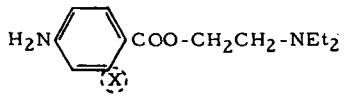
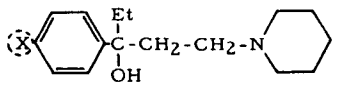
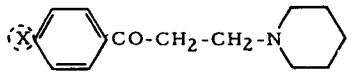
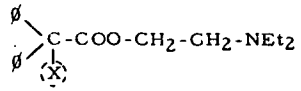
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Local anesthesia minutes (107)	12	15	10			?	9
	Local anesthesia Procaine = 1 (84)			2				4
	Antispasmodic vs. Furmethide Trasentin = ++ (37)		+++	+++				+++
	Ditto (37)		+	0				+
	Antispasmodic vs. acetylcholine, Ba, or histamine (92)			+++		+++		

TABLE 3 (Cont.)

CLASS 1

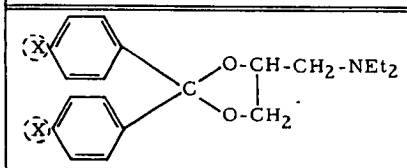
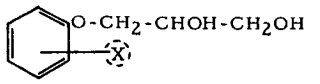
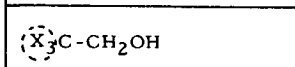
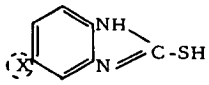
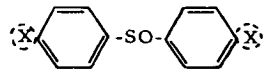
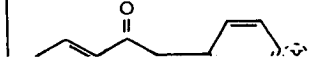
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Antispasmodic vs. acetylcholine, Ba (19)			+				++
	Muscle relaxant Ø-O-CH_2-CHOH-CH_2OH = 1 ortho meta para (9,99)			1.3 1.7 2.4 2.1				2.2 2.1 1.2
	Narcotic (91,101)		strongest	strong				very weak
	Antithyroid Thiouracil = 100 (26)	11	27	44				12
	Insecticidal vs. clothes moth (88)		effective	effective				inactive

TABLE 3 (Cont.)

CLASS 1

Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Antimalarial in ducks ED_{05} in mg/kg	>40 28	29 19	45 20	>60 60			73 15

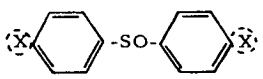
	(26)							
	Insecticidal vs. clothes moth (88)		effective	effective				inactive

TABLE 3 (Cont.)

CLASS 1

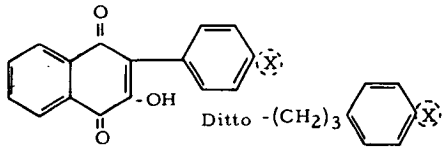
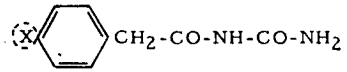
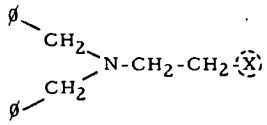
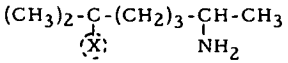
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	Antimalarial in ducks	>40	29	45	>60			73
	ED_{95} in mg/kg	28	19	20	60			15
	(54)							
	Anticonvulsant vs. eleotroshock			±				++
	(136)							
	Adrenergic blocking		active	active		inactive		
	(119)							
	Cardiac action			+		+		
	(100)							
Vitamin D 3-substituent	(10)		inactive	inactive		active		

TABLE 3 (Cont.)

CLASS 1

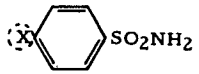
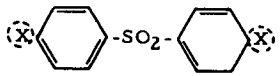
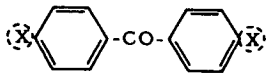
Compound	Biological Test (Reference)	I	Br	Cl	F	OH	NH_2	CH_3
	(88)			insecticidal			bactericidal	
	(88)			ditto			ditto	
	(88)			ditto			ditto (weak)	

TABLE 4

CLASS 1

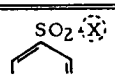
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
				Active vs. bacteria	Active vs. rickettsia

TABLE 4

CLASS 1

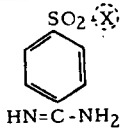
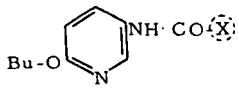
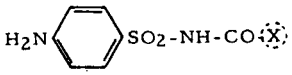
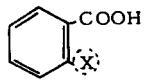
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	(36)			Active vs. bacteria (both have same effect on blood pressure and respiration)	Active vs. rickettsia
	Antitubercular in vitro vs. 607 stasis in mg % (58)			16	1/16
$H_2N\text{-}CS\text{-}NH\text{-}X$	Antithyroid (7)			+	++
$Me_3\overset{+}{N}\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CO\text{-}X$	Parasympathomimetic (117,20)			active	active
	vs. E. coli in vitro Sulfadiazine = 1 (8)			1/125	1/9
	Analgesic (56)		active	inactive	-

TABLE 4 (Cont.)

CLASS 1

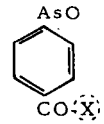
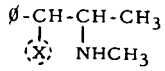
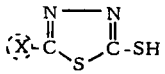
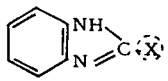
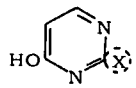
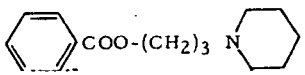
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	vs. T. pallidum in vitro ØAsO = 100 (40)		7	45	
	Pressor CNS stimulation (93)		1 ++	1/3 +	
	Antithyroid Thiouracil = 100 (26, 108)	7		156	
	Ditto	116		6	
	Antithyroid (7)	active	inactive		

TABLE 4 (Cont.)

CLASS 1

Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	Local anesthesia	inactive	active		

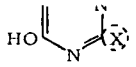
	(7)				

TABLE 4 (Cont.)

CLASS 1

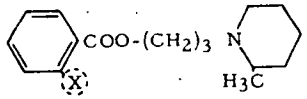
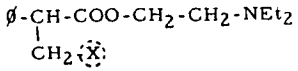
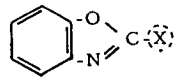
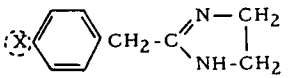
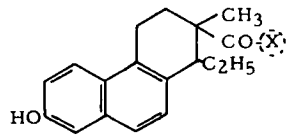
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	Local anesthesia (107)	inactive	active		
	Antispasmodic vs. Acetylcholine Atropine = 1 (21, 63)		1/7		1/10
	Anticonvulsant (25)		inactive		++++
	Effect on blood pressure (130)		pressor		depressor
	Estrogenic (115)		active		inactive

TABLE 4 (Cont.)

CLASS 1

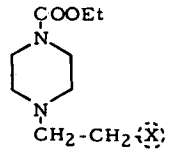
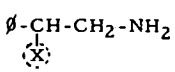
Compound	Biological Test (Reference)	SH	OH	NH_2	CH_3
	vs. Filariasis (139)		inactive		active
	Pressor (66)		1		1

TABLE 5

CLASS 2

Type R-X-R

Compound	Biological Test (Reference)	S	O	NH	CH_2

TABLE 5

CLASS 2

Type R-X-R

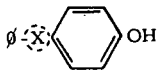
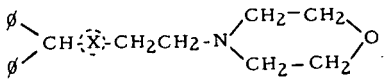
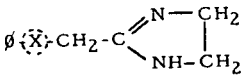
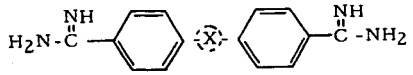
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Phenol Coefficient vs. Staph. aureus (140)	S 100 Se 100	40	10	100
	Antitubercular Salicylate No. (16)		40	2	160
	Antihistamine (17, 97, 127)	weak?	active	very weak	active
	Blood pressure effect (130)		pressor	pressor	depressor
	Trypanocidal (6)	+ -	+++	+	++

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

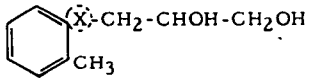
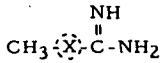
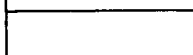
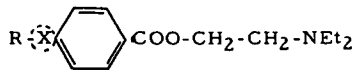
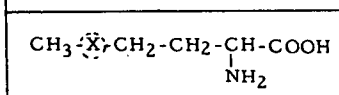
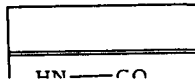
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Muscle relaxant Ø-O-CH_2-CHOH-CH_2OH = 1 (99)	1.3	2.2	0.3	
	Pressor effect (51)	++	+	+	+
	Narcosis (56)	inactive	narcotic	inactive ?	narcotic
	Local anesthesia (75, 118)	inactive	active	active	
	Lipotropic activity (133)	active	active		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antivonvulsant	+++	+++		++++

NH_2	(133)	active	active		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

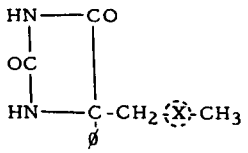
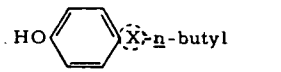
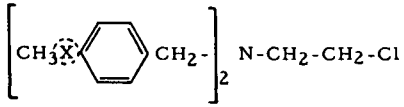
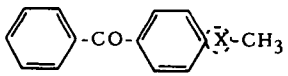
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antivonvulsant vs. electroshock (98)	+++	+++		++++
	Estrogenic (135, 106)		100 mg.		100 mg.
	Phenol coefficient vs. Staph. aureus (140)	60	9		20
	Adrenergic blocking (119)		++		inactive
	Antitubercular stasis in mg % (57)		1.7		20

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

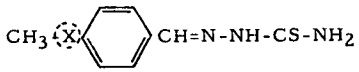
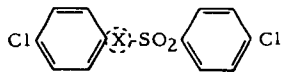
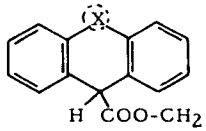
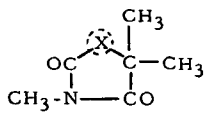
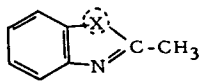
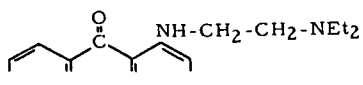
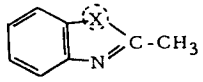
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antitubercular in mice (74)	slight	active	slight	
	Insecticidal (88)		active	inert	
	Antispasmodic vs. acetylcholine Atropine = 1 vs. Ba Papaverine = 1 (22)	1/45 3/4	1/3 3	1/21 3/4	1/35 1.5
	Anticonvulsant (136)	inactive	active	inactive	
	Anticonvulsant (25)	very little	active		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

Compound	Biological Test (Reference)	S	O	NH	CH_2
	Schistosomiasis in humans	active	inactive		



(25)

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

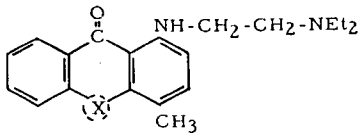
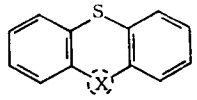
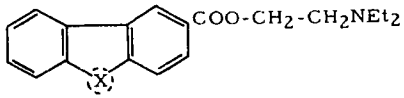
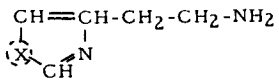
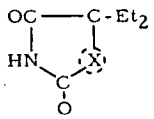
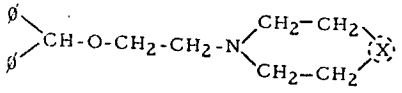
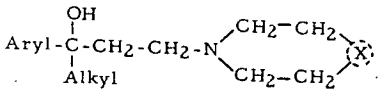
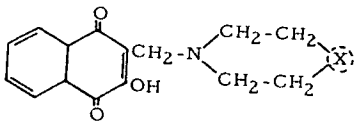
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Schistosomiasis in humans in mice (12)	active active	inactive active		
	Insecticidal (113)		active	active	
	Local anesthesia rabbit cornea (23)	+	++	+++	
	Effect on blood pressure (49)	depressor		depressor	
	Narcotic (47)	++	+		

TABLE 5 (Cont.)

CLASS 2

Type R-X-R

Compound	Biological Test (Reference)	S	O	NH	CH_2
	Antihistamine Benadryl = 1 (97)	weak ?	1/2	inactive	1
	Antispasmodic (37)		+		++
	Antimalarial in ducks (54)		inactive		active

Aromatic rings are treated under "Special Cases".

TABLE 6

CLASS 2

X
‖
Type R-C-R

Compound	Biological Test (Reference)	S	O	NH	CH_2
CO——NH	Hypnotic				

TABLE 6

CLASS 2

Type $R-\overset{\overset{X}{\|}}{C}-R$

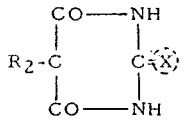
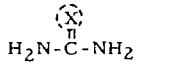
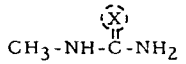
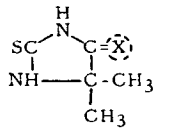
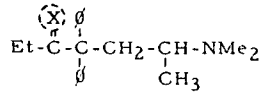
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Hypnotic effect duration (32, 141, 142. 56, 116)	 ++ +	 + ++	 inactive	 weak
	Diuretic (96)	1	1	(toxic)	
	Ditto (96)		0. 8	13. 4	
	Anticonvulsant % protection from metrazol (68)	100%	44%	0	
	Analgesic mg/kg (29)		12. 5	12. 5	

TABLE 6 (Cont.)

CLASS 2

Type R-C(=X)-R

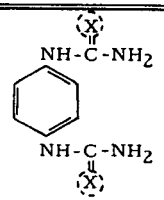
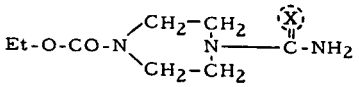
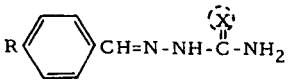
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Trypanosomiasis (71)		inactive	active	
	Filariasis (86)		inactive	active	
	Antitubercular in mice (74)	active	inactive	inactive	

TABLE 7

CLASS 2

Type R-CO-X-R

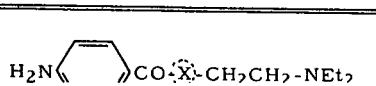
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Local anesthesia	active	active	weak?	

TABLE 7

CLASS 2

Type R-CO-X-R

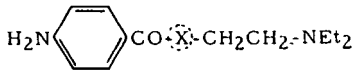
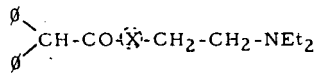
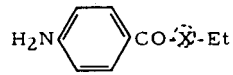
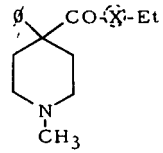
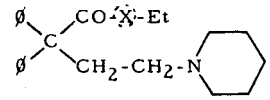
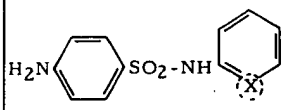
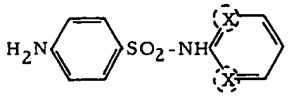
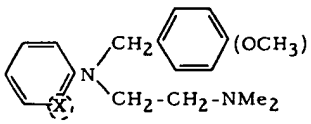
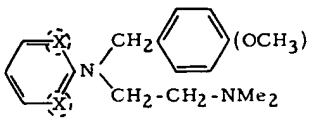
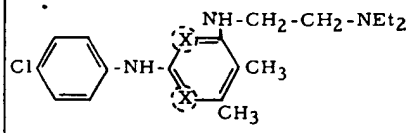
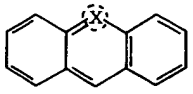
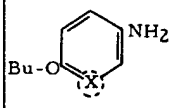
Compound	Biological Test (Reference)	S	O	NH	CH_2
	Local anesthesia (118, 65)	active	active	weak?	
	Antispasmodic vs. acetylcholine Trasentin = 1 (21, 110, 124)	4	1	7/10	
	Antitubercular salicylate no. (16)		40	1	80
	Analgesic (84)		1		1
	Analgesic (84)		1		1/5

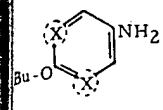
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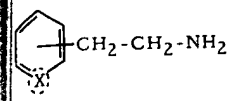
CLASS 3

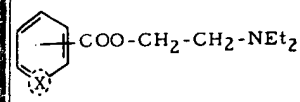
Aromatic Rings

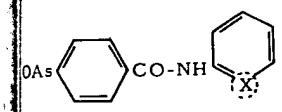
Compound	Biological Test (Reference)	=CH-	=N-
	Antibacterial (8, 120)	moderate	active
	Ditto	moderate	very active
	Antihistamine (148, 78, 80, 59)	active	active
	Ditto	active	active
	Antimalarial (102)	inactive	active
	Antispasmodic vs. acetylcholine Atropine = 100 (92)	15	5
	Antitubercular vs. 607 mg. % stasis (52)	1/16	1/32

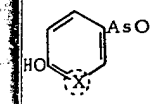
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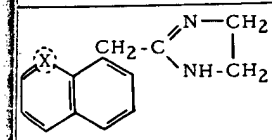












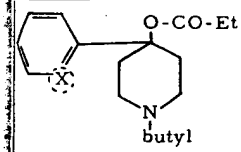


TABLE 8 (Cont.)

CLASS 3

Aromatic Rings

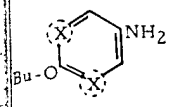
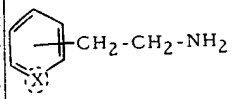
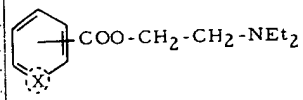
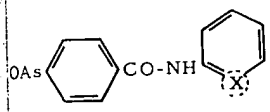
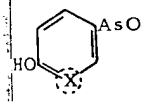
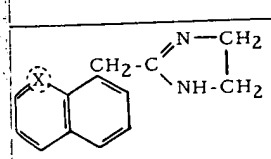
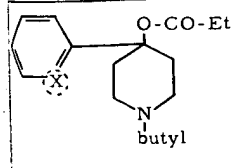
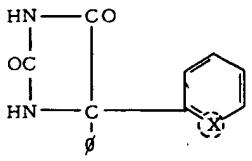
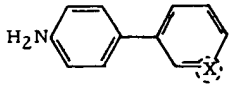
Compound	Biological Test (Reference)	=CH-	=N-
	Antitubercular vs. 607 mg. % stasis (52)	1/16	1/16
	Effect on blood pressure (66, 77, 60)	pressor	α depressor β pressor γ pressor (weak)
	Local anesthesia (30)	active	α inactive β active (weak) γ inactive
	vs. T. pallidum in vitro ØAsO = 100 (41)	97	74
	Syphilis (56, 123)	active	active
	Effect on blood pressure (130)	pressor	depressor
	Analgesic (125)	strong	weak

TABLE 8 (Cont.)

CLASS 3

Aromatic Rings

Compound	Biological Test (Reference)	=CH-	=N-
	Anticonvulsant vs. electroshock (98)	++++	++++
	Antitubercular salicylate no. (44)	1600	600

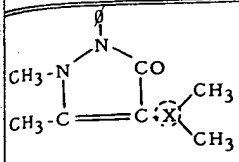
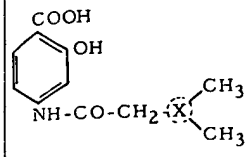
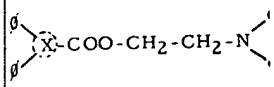
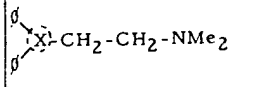
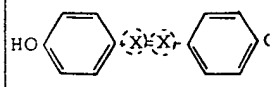
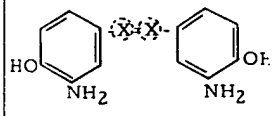
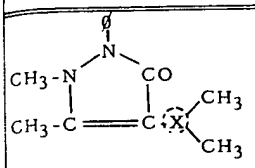
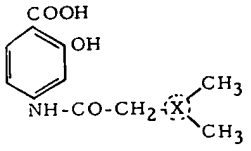
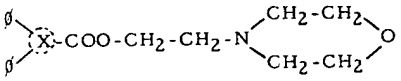
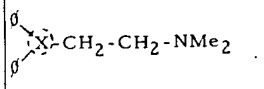
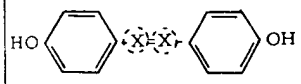
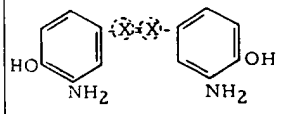
Compound







TABLE 9

CLASS 3

Compound	Biological Test (Reference)	=CH-	=N
	Antipyretic (50, 121)	+	+
	Antitubercular stasis at mol. conc. (72)	1/1200	1/160
	Antispasmodic intestinal strip Papaverine = 100 (28)	75	30
	Antihistamine Benadryl = 1 (126, 104)	1	1.25
	Estrogenic (131, 135)	10 mg.	10 mg.
	Syphilis (149)	Sb unstable	As Salvarsan

=CH-	=N-
-+++	++++
1600	600

TABLE 10

CLASS 4

Compound	Biological Test (Reference)	C	N	S	P	As	Sb
$Me_{3,4}\overset{+}{X}$	Muscarine-like (depressor) (76)		+++	++	++	+	+
$Me_{2,3}\overset{+}{X}-CH_2-CH_2-OAc$	Depressor Acetylcholine = 100% (151, 122)		100	10	10	2	
$Me_3\overset{+}{X}-CH_2-CH_2-OH$	Lipotropic activity Methyl donor (150)		+ yes			+ no	
$Me_3\overset{+}{X}-CH_2-CH_2-OAc$	Rate of hydrolysis by cholinesterase (2)	fast	fast				

TABLE 11

SPECIAL CLASS

Aromatic C=C, S

Compound	Biological Test (Reference)	-HC=CH-	S	O	NH
 $COO-CH_2-CH_2-NEt_2$	Local anesthesia Cocaine = 10	4	1	trace	2

TABLE 11

SPECIAL CLASS

Aromatic C=C, S

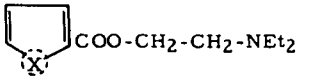
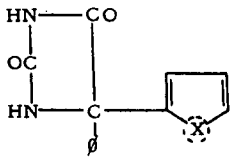
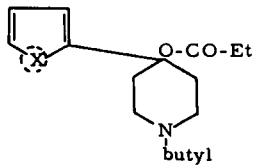
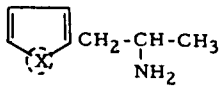
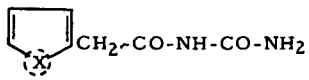
Compound	Biological Test (Reference)	-HC=CH-	S	O	NH
	Local anesthesia Cocaine = 10 (62)	4	1	trace	2
	Anticonvulsant (98)	++++	++++		+
	Analgesic (125)	strong	weak	weak	
	Pressor in dogs stimulatory in humans (4)	++ +	++ 0	+ 0	
	Anticonvulsant Electroshock Metrazole (136)	 +++ ++	 + 0	 0 0	

TABLE 11 (Cont.)

SPECIAL CLASS

Aromatic C = C, S

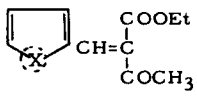
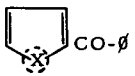
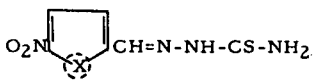
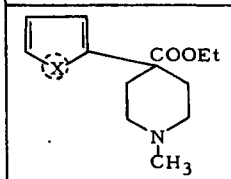
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Insecticidal vs. clothes moth (88)	excellent		excellent	
	Antitubercular mg. % stasis (57)	5		10	
	Antitubercular in mice (74)	active		inactive	
	Analgesic (13)	++		+	
	Antispasmodic vs. acetylcholine Atropine = 1 (15,53)	1/40	1/20		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C= C, S

Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Antihistamine, ileum PBZ = 1	1	1	1	

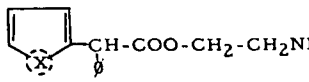
	Antispasmodic vs. acetylcholine Atropine = 1 (15,53)	1/40	1/20		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C= C, S

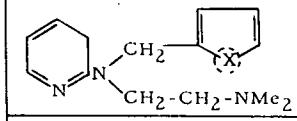
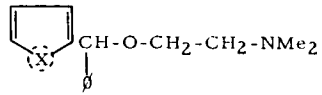
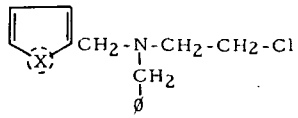
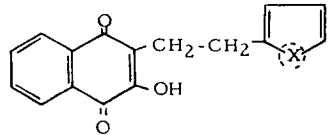
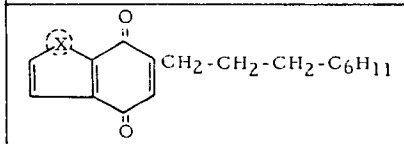
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Antihistamine, ileum PBZ = 1 (104,80,89,11)	1	1	1	
	Ditto Benadryl = 1 (97, 104, 11)	1	1/4	ca. 2	
	Adrenergic blocking (119)	++	++		
	Antimalarial in ducks ED_{95} in mg/kg (54)	>100	85		
	Ditto (54)	20	weak		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C = C, S

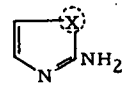
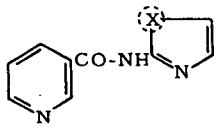
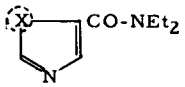
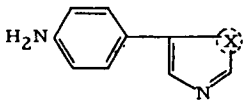
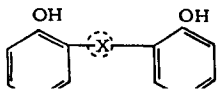
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Antithyroid (132)	0	++		
	Antitubercular ++ corresponds to 50% of streptomycin activity (87)	0	++		
	Analeptic (48)	+	+		
	Antitubercular salicylate No. (44)	600	2,000		
	Antibacterial (120)	inactive ?	weak		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C = C, S

Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Fungicidal	++	++		

	(44)				
	Antibacterial (120)	inactive ?	weak		

TABLE 11 (Cont.)

SPECIAL CLASSES

Aromatic C = C, S

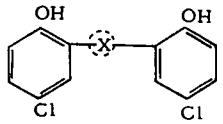
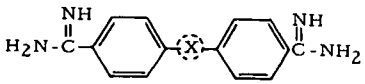
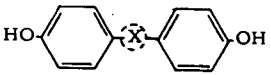
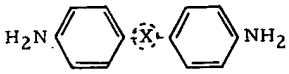
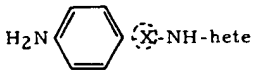
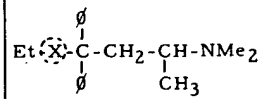
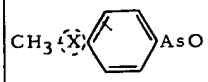
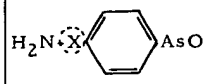
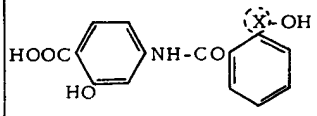
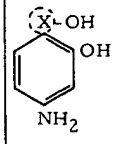
Compound	Biological Test (Reference)	-CH=CH-	S	O	NH
	Fungicidal (105)	++	++		
	Trypanocidal mice (6)	+++	+	+++	++
	Estrogenic dose in mg. (135, 106)	10		100	

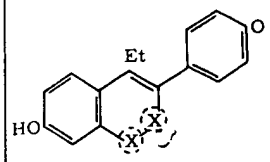
TABLE 12

SPECIAL CLASS

CO, SO, SO_2

Compound	Biological Test (Reference)	CO	SO	SO_2
	Insecticidal vs. clothes moth (88)	inactive	active	active
	Antibacterial vs. pneumococcus (120)	inactive	weak	active
	Antibacterial (83,120)	inactive		active
	Analgesic Amidone = 100 (42)	100		120
	vs. T. pallidum ØAsO = 100 (39)	42		49
 HO- " Me_2N- "	vs. T. pallidum ØAsO = 100 (41)	45 7 8		29 3 112
	Antitubercular in vitro (72)	active		inactive
	Ditto (94)	active		inactive

Compoun



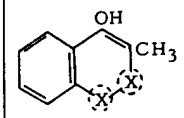
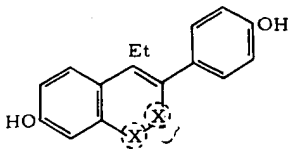
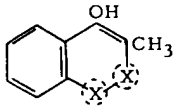


TABLE 13

SPECIAL CLASS

-COO-, CH_2-CH_2

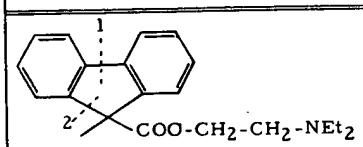
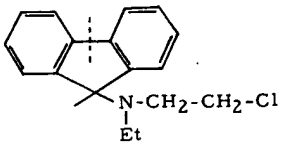
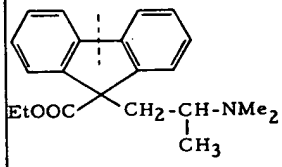
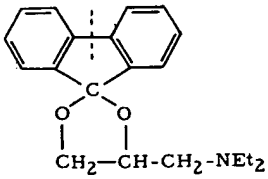
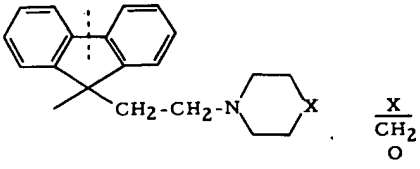
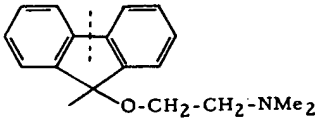
Compound	Biological Test (Reference)	-CO-O	-CH_2-CH_2-
	Estrogenic (112)	100 gamma 	100 gamma
	Vitamin K activity (112)	active 	active

SO	SO_2
tive	active
eak	active
	active
	120
	49
	29 3 112
	inactive
	inactive

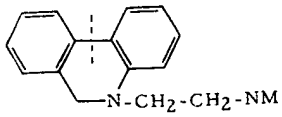
TABLE 14

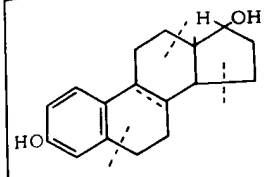
SPECIAL CLASS

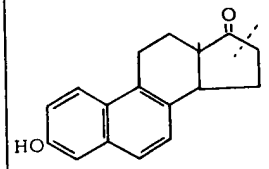
Spatial Fixation by Ring or Double Bond

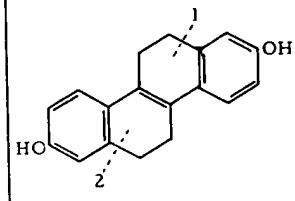
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antispasmodic vs. acetylcholine Atropine = 100 (22,92)	1 2.3 2 0.7	14
	Adrenergic blocking (119)	++	+++
	Analgesia (134)	strong	weak
	Antispasmodic vs. acetylcholine (19)	active	weak
	Antispasmodic vs. acetylcholine, Ba, histamine (84,17)	active	less active
	Antihistamine (126)	active	inactive

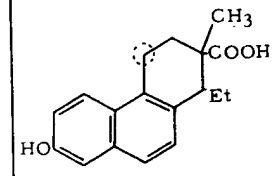
Compound











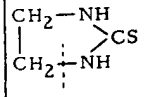


TABLE 14 (Cont.)

SPECIAL CLASS

Spatial Fixation by Ring or Double Bond

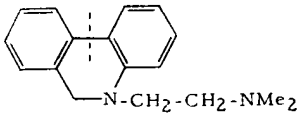
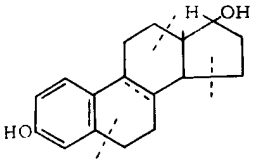
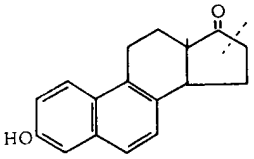
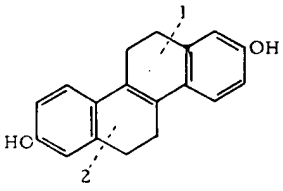
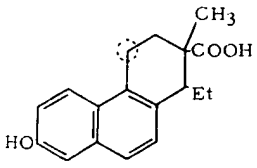
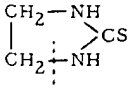
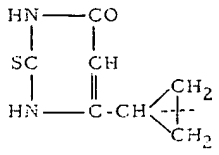
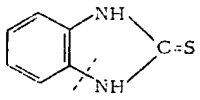
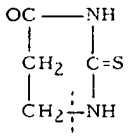
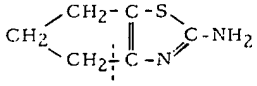
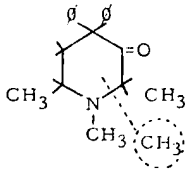
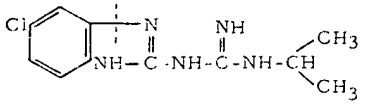
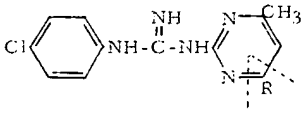
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antihistamine (79)	active	inactive
	Estrogenic (31)	1/40,000 of stilbestrol	active
	Estrogenic in gammas (115)	0.4	1
	Estrogenic (135)	1 ++ 1 and 2 +++	+
	Estrogenic in gammas (115)	0.5	0.4
	Antithyroid Thiouracil = 100 (26)	35	63

TABLE 14 (Cont.)

SPECIAL CLASSES

Spatial Fixation by Ring or Double Bond

Compound	Biological Test (Reference)	Open Form	Ring Form
	Antithyroid Thiouracil = 100 (137)	100	100
	Antithyroid Thiouracil = 100 (109)	14	116
	Ditto (109)	14	10
	Effect on blood pressure (45)	depressor	pressor
	Analgesic (14)	active (Amidone)	inactive
	Antimalarial (1)	active (Paludrine)	inactive
	Antimalarial (34)	+++	++

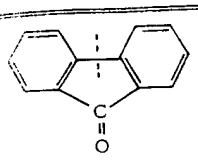
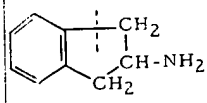
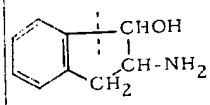
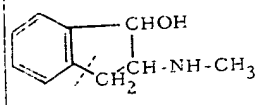
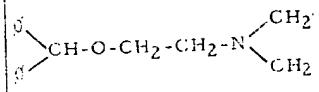
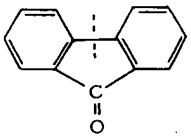
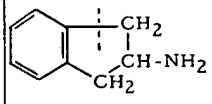
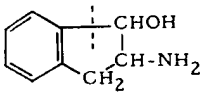
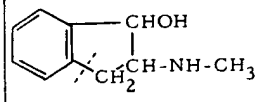
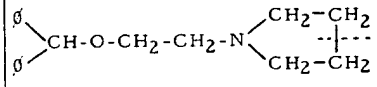
Compound






TABLE 14 (Cont.)

SPECIAL CLASS

Spatial Fixation by Ring or Double Bond

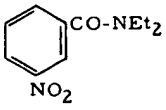
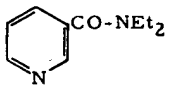
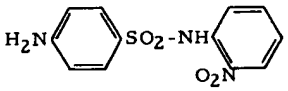
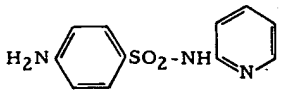
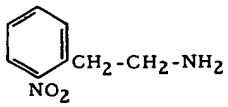
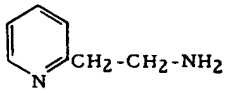
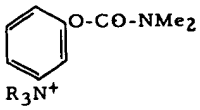
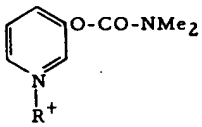
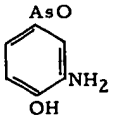
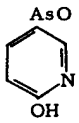
Compound	Biological Test (Reference)	Open Form	Ring Form
	Antitubercular (57)	active	inactive
	Sympathomimetic Vasoconstrictor Pressor (66)	+ weak	+ weak
	Ditto Vasoconstrictor Pressor (66)	+ +	+ weak
	Bronchodilator (69)	good	good
	Antihistamine Benadryl = 1 (153)	0.1	2

m	Ring Form
	100
	116
	10
r	pressor
)	inactive
)	inactive
	++

TABLE 15

SPECIAL CLASS

Polarity Shift

TEST	Exocyclic	Endocyclic
Analeptic activity (46)	 Active	 Active
vs. Hem. strep. in mice (43)	 15% greater activity than Sulfanilamide	 (Sulfapyridine)
Histamine-like activity (90)	 None (pressor compound)	 Weak (0.02 x histamine)
Parasympathomimetic (154, 73, 3)	 Active	 Active
vs. Syphilis (123)	 Active	 Active

TEST
Antibacterial vs. Staph. aureus (146)
Antibacterial (145)
Local anesthetic (30)
Analgesic (129)
Vit. K. activity (114)

TABLE 15 (Cont.)

SPECIAL CLASS

Polarity Shift

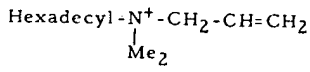
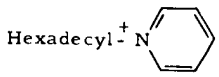
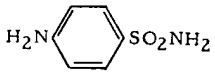
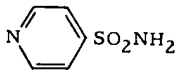
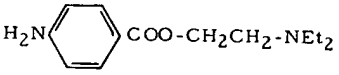
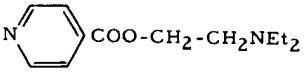
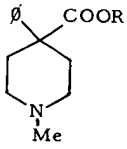
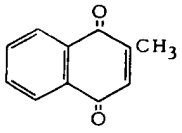
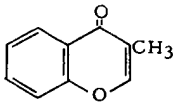
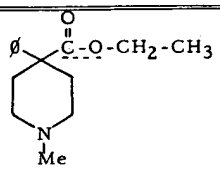
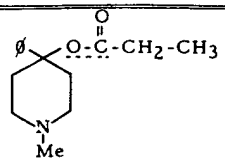
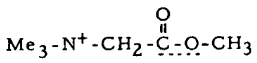
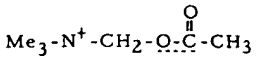
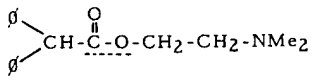
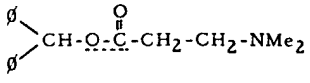
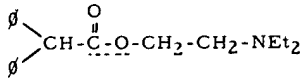
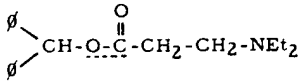
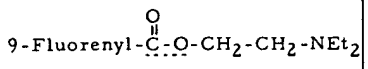
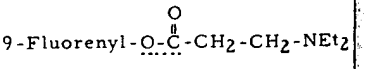
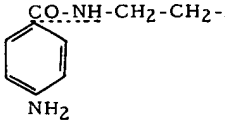
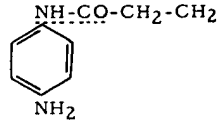
TEST	Exocyclic	Endocyclic
Antibacterial vs. Staph. aureus (146)	 1/25,000	 1/25,000
Antibacterial (145)	 Active	 Inactive ?
Local anesthetic (30)	 Active	 Inactive
Analgesic (129)	 Weak	 Strong
Vit. K. activity (114)	 Potent	 Weak, but active

TABLE 16

SPECIAL CLASS

Reversed Polarity

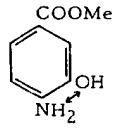
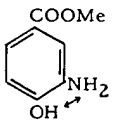
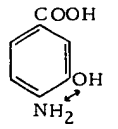
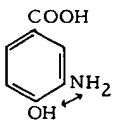
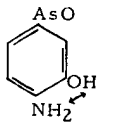
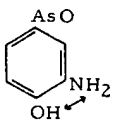
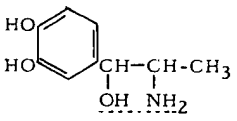
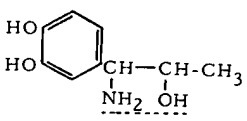
TEST		
Analgesic (82, 55)	 Demerol	 30 x Demerol
Choline-like (Muscarinic) Acetylcholine = +++ (33)	 (Betaine ester) ++	 (Acetyl - formocholine) ++
Antispasmodic vs. Histamine vs. Acetylcholine (5)	 very weak moderate	 very weak moderate
Antispasmodic (35)	 (Trasentin)	 Activity?
Ditto (35)	 (Pavatrine)	 Activity?
Local anesthetic (61)	 weak?	 Activity?

TEST	
Local anesthetic (56)	(Ort
Antitubercular molar conc. for stasis (72)	
vs. T. pallidum in vitro ØAsO = 100 (40)	
Pressor (85, 66)	

TABLE 16 (Cont.)

SPECIAL CLASS

Reversed Polarity

TEST		
Local anesthetic (56)	 (Orthoform) Active	 (Orthoform new) Active
Antitubercular molar conc. for stasis (72)	 1/200	 1/200
vs. T. pallidum in vitro ØAsO = 100 (40)	 40	 38
Pressor (85,66)	 1/12 epi	 Inactive

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DISCUSSION

DR. D. W. WOOLLEY (Rockefeller Institute for Medical Research, New York, New York): This question of isosterism is one I have given considerable thought to, and I would like to tell you how I look at the matter. As Dr. Friedman pointed out, Erlenmeyer gave great impetus to this study, with investigations in the early thirties. He came against a blank wall, though, when the isostere of thiamine was made; that is, a thiazole ring in the vitamin was replaced by a pyridine ring and, contrary to what had been expected (that is, that it would have thiamine activity), it was an exceedingly powerful antagonist; it had the exactly opposite effect from thiamine. This was one of the serious challenges to the idea of isosteric replacement giving rise to biological materials of the same potency, and it still is.

The confusion which seems to reign in the field, and to which Dr. Friedman referred, resides partly in this fact. If in a drug, one makes isosteric replacements, such as the exchange of a pyridine ring for a thiazole, one may obtain a compound of similar qualitative action, although in all respects the two may not be identical. Actual experience showed that drugs of similar activity could be found by making such isosteric changes in existing drugs. This may have been because various members of a series of antimetabolites were being made. If, however, the limit is exceeded and the metabolite rather than the analog is made, an entirely different situation arises.

Among pharmacologists and others as well, hormones have been considered as drugs. Choline, for instance, for years, was thought of as just a synthetic drug, until it was isolated from tissues and gradually shown to be an essential metabolite. The case of adrenalin sometimes can be viewed in the same way. The confusion of drugs with metabolites was therefore profound with the hormones. With the vitamins, however, the matter can be seen more clearly. Because the vitamins are essentially nontoxic, enormous doses can be given to normal animals with no detectable effect. This is not true of many of the hormones.

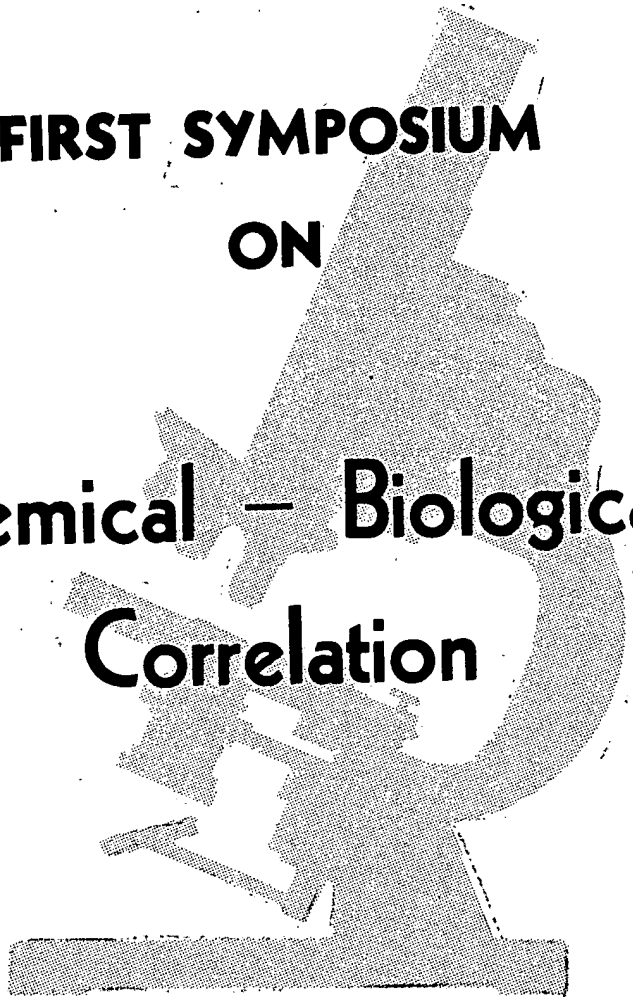
If in a vitamin various isosteric replacements are introduced, an antagonist, frequently a very powerful one, which has exactly the opposite effect to that of the vitamin, may result. The same is true with many amino acids. With this in mind, we can regard some of the groups of isosteric drugs as being mere families of antimetabolites. If the real metabolites to which they may be related are tested, then there should be no surprise if their activity is quite the opposite of the series to which they are isosteric.

I think, in coming down to the practical matter of thinking about synthetizing useful agents, we must never lose sight of this fact that, if we start with the biologically active compound (that is, the metabolite), it is an entirely different problem than if we start with the product in the laboratory; to wit, an already existing analog. Isosteric replacement may have quite opposite effects in these two situations.

DR. FRIEDMAN: This may be an erroneous way of looking at it, but I always consider that an antimetabolite really has the same mechanism of action as the metabolite. Thinking in a pictorial manner, both the metabolite and its isostere start with the same mode of action: the isostere is hooked into the system in the same place as the metabolite would have been. But the rest of the mechanism beyond this stage is blocked when the antimetabolite is present and thus growth, or whatever consequence is involved, is prevented.

FIRST SYMPOSIUM ON

Chemical – Biological Correlation



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The Chemical-Biological Coordination Center is affiliated directly with the Executive Board of the National Research Council of which Mr. William W. Rubey is Chairman. It was established on July 1, 1946, and succeeded the O.S.R.D. Insect Control Committee. Its objectives are: (1) To assemble and organize information which describes (a) the effect of chemicals on biological systems (plants and animals, their organs, tissues, cells and cell constituents); (b) the metabolism of test chemicals within biological systems; (c) the mechanism of drug action or which provides information leading to an understanding of such action. (2) To sponsor the preliminary testing ("screening") of compounds, solicited by the Center, on a variety of plants and animals (including microorganisms) to determine the biological effects of the compounds and to make the resulting data available for use. (3) To prepare reviews of the literature on: (a) The effect of chemical structure upon various biological actions; (b) Test methods used in determining such actions. (4) To sponsor symposia concerned with the correlation of chemical structure with biological activity.

The data in the files of the Center are available to representatives of the sponsoring agencies, the screening agencies and to authorized scientists generally upon request. The sponsoring agencies are the Atomic Energy Commission, the Department of the Army, the Department of the Navy, the U.S. Public Health Service and the American Cancer Society.

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